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MHPG AND DEPRESSION

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Previously we have reported that MHPG is not a predictor of response to antidepressant drugs and we had hypothesized that MHPG levels may essentially reflect a neuro regulatory disturbance in depressed patients and that with evolution of the illness there is a tendency towards regression to the mean of MHPG in the urine. (1 μg/mg creatinine). We have now attempted to test this hypothesis in another study involving 9 patients who were maintained on a VMA Exclusion diet (MHPG control) and given viloxazine up to 450 mg daily over a four week period. MHPG levels were determined in 24 hour urine samples prior to, at 3 weeks and at 4 weeks of drug administration. 8 of the 9 patients had an MHPG level below 1 μg/mg creatinine and showed an increase. Least increase was noted in patients whose pretreatment MHPG levels were closest to 1 μg/mg creatinine. One patient had a pre-drug MHPG level above 1 μg/mg creatinine and showed a decrease in her MHPG level, both at week 3 and week 4. These findings tend to confirm our hypothesis.

NEUROENDOCRINE INVESTIGATION OF HEALTHY VOLUNTEERS ON NORTRIPTYLINE AND LITHIUM

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We have been studying the neuroendocrine and neurotransmitter effects of selected antidepressant procedures. Preliminary results of the first stage of these studies are reported: two groups of healthy male volunteers were given lithium and nortriptyline respectively, in therapeutic doses for 3 weeks, and tested with a neuroendocrine battery prior to and on termination of treatment. Nortriptyline was given in a dosage of 125 mg. a day and lithium in the daily amount to achieve serum lithium levels above .7 mEq/l. The response of TH, TRH, LH, TSH and cortisol to insulin-induced hypoglycemia and to the TRH-LHRH infusion was investigated.

Nortriptyline produced no change in growth hormone and prolactin response to hypoglycemia, however, prolactin response to TRH-LHRH was profoundly increased. Lithium administration produced a profound reduction of growth hormone and prolactin responses to hypoglycemia, but had no effect on the prolactin stimulation by TRH-LHRH. The corresponding data from patients treated in the same manner are compared. The interpretation of these findings is offered and related to the changes in neurotransmitters which control the hormones under study.

CLINICAL RESPONSE, PLASMA LEVELS AND PHARMACOKINETICS OF DESIPRAMINE IN DEPRESSED PATIENTS

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Correlation between clinical response and plasma levels was investigated in seven patients with endogenous depression treated with 2 x 1 mg/kg/day of desipramine (DMI) for 21 days. Clinical response was measured by reduction in Hamilton Depression Rating Scale (HDRS) scores. A beneficial effect of DMI was seen in five out of seven patients studied and was fully evident one week after the beginning of treatment. Great inter-individual differences were observed in DMI plasma levels both after a single dose and at 'steady state'. The maximum plasma concentration after a single dose (C_{max}) ranged between 19 and 179 ng/ml and the mean 'steady state' concentrations between 65 and 240 ng/ml. A curvelinear relationship ($r = 0.969$; $p < 0.001$) was found between plasma levels of DMI and amelioration scores at the end (22nd day) of treatment. Post 'steady state' plasma disappearance half-lives of DMI calculated in four patients ranged from 11.5 to 34.3 hr (mean±S.E.M. = 26.2±5.0). The mean apparent volume of distribution of the drug at 'steady state' in these patients was 17.9 L/kg (10.5 - 26.3 L/kg) and the apparent plasma clearance 0.5 L/kg/hr (0.35 - 0.61 L/kg/hr). The results confirm the interpatient variability in plasma levels of DMI and suggest that a curvelinear relationship may exist between the concentration of the drug in plasma and clinical response.

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POTENTIATION OF CLOMIPRAMINE BY TRYPTOPHAN IN THE TREATMENT OF AGORAPHOBIC AND SOCIAL PHOBIC PATIENTS

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A number of workers have reported a potentiating effect of tryptophan on the recovery of patients treated with MAOI. Recently tryptophan had significantly potentiated the effect of Clomipramine in the treatment of depression and anxiety. Since Clomipramine is reported to be effective in the treatment of agora and social phobic patients, this study evaluates the degree of potentiation of Clomipramine by Tryptophan in the treatment of these groups of patients. This is a double blind 10 week study of 24 agoraphobics and 16 social phobics comparing Clomipramine Tryptophan (C-T) and Clomipramine Placebo (C-P). All patients were placed on a divided fixed changing dosage schedule of Clomipramine starting at 75 mg a day and rising to 200 mg/day. Either tryptophan 3-8 grams a day was concurrently prescribed or an identical placebo. After 8 weeks on either the C-T or C-P régime, the Tryptophan or placebo was withdrawn and a final assessment was made on week 10. Results of the study indicated that in the psychiatric assessment of the phobia (Psychiatric Status Questionnaire for Phobic Illness) and in the Phobic Questionnaire Scale there was significant improvement in all groups but no difference among them. Assessments of other psychopathology revealed that on the BPRS agoraphobics whether on C-T or C-P improved significantly more than social phobics on C-P. On the HAM-D agoraphobics on C-P significantly improved more than social phobics on C-P. On both the BPRS and HAM-D only the social phobics on C-T failed to improve over the course of the study. The psychometric and self-assessment scales revealed no significant difference between groups on the IPAT, Social Adjustment, Self-Assessment Scale. On the Fear Survey schedule social phobics on C-T or C-P had a significantly greater improvement than agoraphobics on C-P. There were significantly more patients who regressed after Tryptophan was withdrawn than when placebo was withdrawn.

This study indicates that Clomipramine with or without tryptophan has a greater effect in the treatment of agoraphobic patients than social phobic patients. The drug appears to act on the symptoms of anxiety and depression as well as on the target phobic symptoms.

EVIDENCE THAT A TRACE AMINE IS ESSENTIAL FOR THE OPERATION OF A BEHAVIOR-CORRELATED RETICULAR FORMATION INPUT TO THE NEOCORTEX

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Normal rats display low voltage fast activity in the neocortex during a variety of behaviors. However, following a large dose of atropine, large amplitude slow waves occur during immobility, tremor, tooth-chattering, and face-washing (Type II behavior) while atropine-resistant low voltage fast activity (ARLVFA) is correlated with the occurrence of walking, struggling, postural changes and head movement (Type I behavior). This effect suggests the existence of 2 components of the ascending reticular formation: 1) a cholinergic component active during a variety of behaviors, and 2) a non-cholinergic component whose activity is coupled to Type I behavior. (Vanderwolf, C.H., Kramis, R., & Robinson, T.E. Pp 199-226 in: Ciba Foundation Symposium 58 (new series) *Functions of the septo-hippocampal system*. Amsterdam: Elsevier, 1978). ARLVFA (due to the non-cholinergic component) is abolished by prior treatment with reserpine (i.e. reserpine plus atropine totally abolish low voltage fast activity) but not by a variety of drugs known to block the synthesis or post-synaptic effects of catecholamines, serotonin, or histamine. Since the effect of reserpine on ARLVFA is blocked by prior treatment with nialamide it is likely that the unknown reserpine-sensitive factor essential to ARLVFA is a substrate of brain monoamine oxidase.

An attempt was made to restore normal ARLVFA in reserpinized rats by administration of various monoamines, their precursors or other compounds. Such experiments should help identify the unknown monoamine involved in ARLVFA.

Rats with chronically implanted electrodes (100μ diameter) were treated with reserpine (10 mg/kg). After a 12-20 hr delay, records were taken of neocortical slow wave activity and behavior using a polygraph and a movement-sensing device. Test compounds were administered either before or after treatment with atropine (50 mg/kg) to eliminate cholinergic activation of the neocortex.

5-Hydroxytryptophan (100-200 mg/kg) did not reverse reserpine-induced catalepsy and akinesia and did not restore ARLVFA. l-Dopa (150-300 mg/kg plus benserazide, 50 mg/kg), apomorphine (.25-2.5 mg/kg) and clonidine (.5-1.0 mg/kg) reversed the catalepsy and akinesia but did not restore ARLVFA. β-Phenylethylamine (PEA, 20-80 mg/kg) was effective in restoring of ARLVFA as well as in reversing catalepsy and akinesia. Restoration of ARLVFA by PEA was not antagonized by α-methyl-p-tyrosine or by catecholamine receptor blockers. Pargyline (50 mg/kg) also rapidly restored ARLVFA in rats pretreated with reserpine.

Reserpine has an effect on reticulo-cortical activity which is not reversed by catecholamine agonists but is easily reversed by a trace amine, PEA, or by pargyline. A trace amine may play an essential role in a component of the ascending reticular formation whose activity is closely correlated with behavior.

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SELECTIVE CHANGES IN BRAIN AMINES AFTER DELAYED RESPONSE (DR) AND SUCCESSIVE DISCRIMINATION (SD) LEARNING IN CATS

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In recent years, studies using lesions or biochemical manipulations have indicated an important role for monoamines in learning. These results show that learning is sensitive to monoamine manipulation, but provide only indirect evidence that learning depends upon monoaminergic processes. The present study was undertaken to show if in intact, undrugged cats learning is accompanied by changes in monoamine concentrations, turnover rates and synthetising enzyme activities. The SD situation was a 2-choice successive learning, implicating the association of a color with the direction of the response whereas the DR situation was a visual discrimination learning with interposed delays (0, 9, 27 and 54 sec). A modified WGTA apparatus was used for conditioning. Training was continued until animals showed significantly correct performance: criterion fixed at 95%. In order to properly interpret observed changes, 2 control groups were used: one non-manipulated baseline group, and one manipulated no-learning group that was put through the experimental procedure but did not learn the task.

Biochemical assays, done on different structures in the brain showed that no difference in biogenic amines and enzyme activity was observed between baseline and manipulated controls. Cats trained on a DR task showed a selective increase of the serotonin content in the piriform lobe, mesencephalon without raphe nuclei and medulla without raphe nuclei whereas in a SD task a significant increase was observed in the neostriatum as well as in the mesencephalon without raphe nuclei. The 5-hydroxyindoleacetic acid content followed the increase in serotonin level except in the pons deprived of its raphe nuclei, where a significant decrease was shown in DR trained cats. The noradrenaline content was significantly decreased in the piriform lobe, thalamus and hypothalamus and increased in the neostriatum of cats trained on a SD learning whereas in a DR task the diminution of the noradrenaline content was restricted to the frontal cortex and piriform lobe. The serotonin and noradrenaline changes were mirrored by an increase in the tryptophan hydroxylase and dopamine-β-hydroxylase activities in the same regions except for the neostriatum where a significant decrease in dopamine-β-hydroxylase activity of the SD trained animals was demonstrated when controls and manipulated cats are pooled together. The present findings revealed that these two learning situations have in common the reactivity of the serotoninergic metabolism in the brainstem but contrast markedly by the preponderant involvement of the NA metabolism in the SD situation.

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BEHAVIORAL AND BIOCHEMICAL EFFECTS OF TRYPTOPHAN, TYROSINE AND PHENYLALANINE IN MICE

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The aromatic amino acids tryptophan, tyrosine and phenylalanine are all precursors of the biogenic amines. Tryptophan administration increases brain 5-hydroxytryptamine (5HT) synthesis and recent studies indicate that tyrosine administration increases noradrenaline synthesis. The purpose of this study was to look at the behavioral and biochemical effects of the three aromatic amino acids in order to obtain more information on their potential therapeutic use in neuropsychiatric disorders.

Behavior was observed in treated mice which were placed either in an open field, or in water in a narrow cylinder. Animals placed in the water (swim test) soon assume a characteristic immobile posture. Porsolt et al., have shown that most antidepressants, including pharmacologically atypical ones, decrease immobility in the swim test and decrease or do not affect activity in the open field test. However, this test is not specific for antidepressants as anticholinergic and antihistaminergic drugs also decrease activity in the swim test, and among the antidepressant drugs the test is relatively insensitive to agents which act selectively on 5HT.

Tryptophan increased immobility in the swim test, and thus did not behave like a normal antidepressant. Recent clinical studies suggest that tryptophan can be an effective antidepressant when given in an appropriate manner, but it is also an antimanic agent. Thus, its behavioral profile is unlike that of other antidepressants in both mice and men. Tyrosine decreased immobility in the swim test and increased activity in the open field test. This pattern of activity was similar to that of the psychostimulants amphetamine and caffeine. Phenylalanine behaved in the mice like an antidepressant; it decreased immobility in the swim test but did not affect open field activity. This was of interest because of two clinical studies by Beckmann et al. suggesting that DL-phenylalanine may have an antidepressant action.

In the biochemical studies we found, as expected, that tryptophan raised brain 5HT, and this presumably accounts for its behavioral effects. Tyrosine did not increase brain catecholamine levels. However, a possible increase in catecholamine turnover would explain its stimulant action in the behavioral test. Phenylalanine raised brain phenylalanine and tyrosine levels but lowered brain dopamine, noradrenaline and 5HT. This may have been due to inhibition of tryptophan and tyrosine hydroxylase by phenylalanine. Presumably the behavioral effects of phenylalanine are not due to changes in brain biogenic amine levels. A possibility is that phenylalanine acts by increasing brain phenylethylamine and/or phenylethanolamine.

Our data indicate that more information is needed on the behavioral and biochemical effects of phenylalanine administration. If phenylalanine is an antidepressant it acts in a different way from tryptophan. Thus a combination of these two dietary components would be worth testing in depressed patients.

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NEURAL COMPONENTS OF THE RESPONSE TO STRESSORS: CENTRAL PATHWAYS MEDIATING THE INDUCTION OF ADRENAL TYROSINE HYDROXYLASE

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Various stressors (e.g. cold, immobilization) lead to an increase in the activity of adrenal tyrosine hydroxylase (ATHA). The synthesis of this new enzyme protein is mediated transneuronally, and does not occur in splanchnicotomized rats. Administration of certain drugs, which presumably act as receptor-stimulating agonists, can influence the increase of ATHA, as can intraventricularly injected neurotoxins. By use of such drugs and by imposition of specifically placed lesions in the brain and spinal cord, it has been possible to delineate portions of the pathways involved in the enzyme induction and the neurotransmitters concerned. The results taken as a whole suggest that more pathways than one exist above the level of origin of the splanchnic nerves for the rise of ATHA. This raises the possibility that different stressors have their effects on peripheral sensory receptors or proprioceptors committed to different neuronal pathways. These effects would ultimately be funnelled through the splanchnic nerves, increasing the activity of those of their fibres mediating the increase of ATHA.

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MOLINDONE ON MOUSE STRIATAL TYRAMINE

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Molindone is an indole derivative with neuroleptic effects in animals and which in humans possesses anti-schizophrenic activity equivalent to that observed for the phenothiazines and butyrophenones. The increase in striatal dopamine turnover induced by anti-schizophrenic drugs has been shown to occur in association with a reduction in striatal p-tyramine (Juorio, Life Sci., 20, 1663, 1977). This is an investigation of the effects of molindone on the concentration of striatal tyramine and homovanillic acid in the mouse. For comparison the effects of fluphenazine on their concentrations was studied also.

Male albino Swiss mice were killed and the striatum was used for the determinations. The amines in the tissue homogenate were derivatized with a dansyl chloride, separated chromatographically and estimated by the high resolution mass spectrometric integrated ion current technique using

deuterated p- or m-tyramine as internal standards. Homovanillic acid was extracted from the tissue homogenate into butyl acetate, transferred to Tris buffer and estimated fluorimetrically.

The subcutaneous administration of low doses of molindone (2 mg/kg, 2 hours) produced a significant reduction (to 64% of controls) in striatal p-tyramine accompanied by an increase (to 181% and 233% of controls) for m-tyramine and homovanillic acid respectively. Similar effects were produced by all doses of fluphenazine (0.1-5.0 mg/kg) employed. By increasing the dose of molindone to 20 mg/kg the striatal concentrations of both p- and m-tyramine were increased (to 120-150% of controls) at 2 hours after drug administration while the concentrations of homovanillic acid were higher than controls but lower than after 2 mg/kg. The highest dose of molindone used (100 mg/kg, 2 hours) produced a statistically significant increase in p-tyramine but did not change m-tyramine. The concentrations of homovanillic acid were higher than the controls (141% of controls) but lower than after 2 or 20 mg/kg.

Low doses of molindone produced effects that are consistent with those observed after blockade of dopamine post-synaptic receptors while at higher doses, it acts as a partial agonist of dopamine receptor sites or a blocker of dopamine pre-synaptic receptors. These results fit well with the proposal of a reciprocal relation between dopamine and tyramine, though it is not possible yet to ascertain if tyramine controls dopamine or vice-versa or if it is a more remote relation.

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SELECTIVE RELEASE OF TRITIATED *p*-TYRAMINE (pTA) FROM RAT STRIATAL SLICES

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We have recently demonstrated that the CNS stimulant, methylphenidate, released the trace amines, *m*-tyramine (mTA) and *p*-tyramine (pTA), to a significantly greater extent than the putative transmitter dopamine (DA). In contrast d-amphetamine released equal amounts of DA, mTA, and pTA. Since reserpine enhanced the methylphenidate release of DA, it seemed reasonable to conclude that methylphenidate could release extragranular amines, as has been generally accepted for amphetamine. The purpose of this study was twofold. First, to determine whether other non-amphetamine-like CNS stimulants also had more pronounced releasing effects with respect to trace amines; and second, to try to determine whether or not the non-amphetamine-like group acted on a different cytoplasmic store of amine.

The simultaneous release of pTA-^{3}H and DA-^{14}C from slices (0.2mm thickness) of rat striatum was studied. The anterior portion equivalent to 10-15 mg wet weight of individual striata was incubated for 15 min with 100 nM of each labelled amine to preload the slices. They were then moved in succession through a series of ten tubes containing a Krebs-Henseleit medium ± the drug to be tested. Pargyline (10μM) was present in all the media, and the drugs were present in tubes (fractions) 6 to 10 inclusive. The amount of radioactive amine released into each fraction was expressed as a percentage of the total of the amounts released into all the fractions plus that left in the slices.

The drug concentrations used were 10μM in all cases except with amphetamine which was used at 1μM either alone or in combination with another drug. The spontaneous releases in fraction 5 and the stimulated release caused by the various drugs in fraction 6 (the highest) are tabulated below (mean ± S.E.M.).

Drug	pTA-^{3}H Release		DA-^{14}C Release	
	Spontaneous	Stimulated	Spontaneous	Stimulated
methylphenidate	3.3±.2	14.4±1.1	3.1±.3	4.7±.3
cocaine	3.2±.5	9.8±1.4	2.1±.2	3.0±.2
nomifensine	3.5±.3	9.5±0.6	2.9±.1	3.2±.2
amfonelic acid	2.7±.2	16.5±1.1	2.0±.3	3.6±.9
d-amphetamine	2.7±.2	7.2±0.8	2.3±.4	11.2±.3
" + methylphenidate	3.4±.5	10.3±1.3	2.6±.5	4.4±.6
" + cocaine	4.1±.5	9.1±1.0	3.0±.7	4.8±.6
" + nomifensine	3.2±.4	8.3±0.6	2.6±.7	3.7±.6

It is evident that the CNS stimulants other than amphetamine selectively release pTA in preference to DA from rat striatal slices. This is suggestive of a role for pTA, and perhaps other trace amines, in the mode of action of these drugs. The combined effects of amphetamine and the other drug were not additive and, in fact, the combination behaved like the non-amphetamine drug alone. These antagonistic effects suggest that different mechanisms are involved in the action of amphetamine than with the other drugs. Such a difference could involve different cytoplasmic pools of amines on which these drugs act.

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A SENSITIVE AND SPECIFIC RADIOENZYMATIC ASSAY FOR NORADRENALINE AND ADRENALINE

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Radioenzymatic assays for the determination of the catecholamines, noradrenaline (NA) and adrenaline (A), were introduced over a decade ago. The most commonly used methods are based on the transfer of a radioactive methyl group from tritiated S-adenosyl-L-methionine (3H-SAM) to the 3-hydroxyl group of the catecholamine molecule by catechol-0-methyl transferase (COMT). The methylated derivative of NA or A (normetanephrine (NMN) or metanephrine (MN), respectively) is then separated and its radioactivity measured. In those procedures in which the derivatives are extensively purified, sensitivities of 10 pg or less have been reported.

The present assay was developed to enable measurement of the very small quantities of NA believed to be released into artificial CSF perfused through the rat hypothalamus. Previous experiments by others in our laboratories had indicated that the dansyl derivative of NMN could be easily prepared and isolated. This paper describes a radioenzymatic assay in which dansyl NMN and MN are used to determine NA and A in the picogram range.

COMT was prepared from rat liver according to published procedures. The tissue was finely minced, washed with isotonic saline, then homogenized in 1.15% KCl. A 78,000 x g supernatant fraction was prepared and adjusted to pH 5 with 1 M acetic acid; COMT was isolated by ammonium sulfate fractionation. The enzyme preparation was dialyzed against 1 mM sodium phosphate buffer, pH 7, and frozen at -70° until use. NA or A samples (1 pg to 50 ng in 300 μl of CSF/0.6 N perchloric acid, 5:1 (v/v) were incubated for 1 hour at 37° with 100 μl of an enzyme solution consisting of 2.8 M tris acetate buffer, pH 9.1, containing 0.32 M $MgCl_2$ (50 μl), COMT solution (25 μl), 0.05 M pargyline (5 μl), 0.05 M EGTA (10 μl), 3H-SAM solution (5 μl) and CSF/0.6 N perchloric acid, 5:1 (v/v) (5 μl). The reaction was stopped by adding 300 μl of 0.5 M borate buffer, pH 10, containing 3 μg of NMN or MN as carrier. The resulting solution was saturated with $NaHCO_3$ and the dansyl derivatives prepared by overnight reaction with dansyl chloride reagent (1 mg/ml in acetone). After extraction of the dansylated products into ethyl acetate, dansyl NMN or MN was isolated by two successive thin layer chromatographic separations and its radioactivity measured.

The assay provides a very sensitive and specific method for determining NA and A; as little as 2 to 3 pg of either amine can be measured. Less than 1% interference has been observed when equal amounts of similar compounds, including dopamine, 3-methoxytyramine, NMN, MN, Dopa, 6-hydroxydopamine, tyramine, tryptamine, phenylethylamine and octopamine were assayed. Because of the brilliantly fluorescent nature of the dansylated compounds and the very narrow bands which they exhibit during thin layer chromatography, the use of this derivative provides a convenient means of detection and determination of the catecholamines, and may also be useful in determining other compounds having similar structures.

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SIGNIFICANCE OF 3,4-DIHYDROXYPHENYLETHYLENE GLYCOL (DHPG) FORMATION IN CNS NOREPINEPHRINE METABOLISM

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While 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) is a major brain NE metabolite, formation of 3,4-dihydroxyphenylethylene glycol (DHPG) may also be a significant pathway of NE metabolism in the brain of some animal species (Braestrup et al., J. Neurochem. 23: 569, 1974;

Karasawa et al., J. Neurochem. 30: 1525, 1978). A variety of data indicate that brain MHPG formation reflects changes in brain NE neuronal activity and release, but the relationship of brain DHPG formation to NE neuronal activity is not well established. Further, significant quantities of DHPG have been demonstrated in human urine by gas chromatography-mass spectrometric assay (GC-MS) (Muskiet et al., Clin. Chem. 24: 2001, 1978). However, the occurrence of DHPG in the central nervous system (CNS) of humans has not been demonstrated as yet.

To investigate these questions a sensitive and specific GC-MS assay has been developed in this center for the simultaneous determination of MHPG and DHPG in biological samples. In this procedure, MHPG and DHPG are assayed as their respective acetyl-trifluoroacyl esters and quantitated by multiple ion mass fragmentography using deuterium labeled MHPG and DHPG as internal standards.

Determination of free and total DHPG and MHPG in rat brain regions demonstrated that DHPG concentrations are equal to or greater than respective MHPG levels in almost every region assayed. In hypothalamus total DHPG (mean ± S.E.M. = 290 ± 19 ng/gm; n =6) concentrations were almost 3 times the levels determined for MHPG (100 ± 6.2 ng/gm; n = 6), suggesting that DHPG formation may be relatively more important than MHPG in rat hypothalamic NE metabolism. In mouse brain, which has very little conjugating capacity for catecholamine metabolites, MHPG and DHPG were almost entirely free. Further, mouse brain MHPG concentrations exceeded those of DHPG, in contrast to the rat. These observations suggest sulphate conjugation of DHPG may be important in determining the extent to which brain NE is cleared through DHPG formation and efflux.

In rats given the α-adrenergic antagonist yohimbine (1-10 mg/kg, i.p.) 2 hr prior to sacrifice, there was a parallel and dose dependent increase in forebrain, hindbrain and hypothalamic DHPG and MHPG concentrations. Five hr following administration of the α-adrenergic agonist clonidine (250 μg/kg, i.p.) there was a parallel decrease (30%) in spinal cord MHPG and DHPG concentrations. These findings suggest rat brain DHPG formation is also sensitive to changes in NE neuronal activity.

The occurrence of DHPG in human CNS is supported by our findings of significant amounts of DHPG (2.17 ± 0.57 ng/ml; n= 6) compared to MHPG (9.92 ± 1.15 ng/ml; n = 8) in CSF of neurological patients. Taken together the above observations support the notion that DHPG formation and outflow is a major route for the metabolic clearance of NE in the CNS of some species. Further, determination of DHPG may be important in the biochemical evaluation of CNS noradrenergic activity.

URINARY EXCRETION OF LABELLED METABOLITES FOLLOWING INGESTION OF DEUTERATED PHENYLETHYLAMINE IN A HUMAN MALE

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Interest in the trace amines is now being extended to their acid metabolites. One of the safest and most effective ways of assessing amine metabolism in humans is to ingest a deuterated precursor followed by the identification and quantitation of its labelled metabolites by mass spectrometry.

In this paper we report the results of an experiment in which a male subject ingested 500 mg phenylethylamine-d_2. Urine collected over the following two 12 hour periods was analysed for free and conjugated deuterated phenylethylamine, *m*- and *p*-tyramine, phenylacetic acid and *m*- and *p*-hydroxyphenylacetic acid. The metabolites were extracted with ethyl acetate, derivatized and analysed by gas chromatography-mass spectrometry in the case of acid metabolites (heptafluorobutyryl/methyl ester or pentafluorobenzyl derivative) and the IIC solid state mass spectrometric procedure in the case of dansyl amines.

The results show that the major metabolite is a conjugate of phenylacetic acid (equivalent to 55.8 mg free phenylacetic acid). All the other deuterated metabolites searched for were found, but in much smaller quantities: 2 to 4 μg of each of the amines (free and conjugate) and 50 to 800 μg of the hydroxyphenylacetic acids (free and conjugate). Although it isn't possible to exclude the possibility of metabolism in the gut, other experiments have indicated that this is extremely unlikely in the case of unconjugated metabolites. The results therefore indicate that hydroxylation of arylalkylamines occurs in the human.

We thank Dr. Juorio and other colleagues in the Division for their help and criticism and Saskatchewan Health and the Medical Research Council of Canada for continuing support.

CHANGE IN RESPONSIVENESS OF RAT CORTICAL NEURONES TO ACETYLCHOLINE FOLLOWING CHRONIC ADMINISTRATION OF ATROPINE, IMIPRAMINE AND VILOXAZINE

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Recent work has shown that long term treatment with antidepressants results in adaptive changes in the sensitivity of monoamine systems in the CNS. When administered on a clinically relevant time scale tricyclic antidepressants cause a decrease in the sensitivity of central noradrenaline systems. Other evidence suggests that at least in some brain areas there is a concommitent increase in sensitivity of 5HT systems. However, although tricyclics are known to be potent blockers of muscarinic cholinergic receptors, little is known of the effects of long term administration of these drugs on central cholinergic systems. These experiments have investigated this problem.

Male Wistar rats received daily injections (IP) of the tricyclic antidepressant imipramine (5 mg/kg) or the non-tricyclic antidepressant viloxazine (5 mg/kg) for 14 days. For comparison a third group of rats received atropine (2.5 mg/kg) for 14 days. Control rats received daily injections of 0.9% saline. Thirty-six hours following the final injection, rats were anaesthetized with urethane and prepared for unit recording coupled with extracellular microiontophoresis. Recordings were made from single, spontaneously active neurones in the somatosensory cortex. The sensitivity of these neurones to iontophoretically applied ACh and L-glutamate was determined.

In rats treated with saline and in untreated rats the vast majority of cortical neurones were excited by iontophoretically applied ACh. Only an occasional superficially located cell exhibited a decrease in firing rate in response to ACh. However in animals pretreated with imipramine, viloxazine or atropine these proportions were completely reversed. In all three groups the predominant neuronal response to ACh was one of depression with excitation occurring much less frequently. In contrast, L-glutamate excited the great majority of neurones in all groups.

Comparison of the mean depths of neurones studied in the saline treated and drug treated groups showed no significant differences. In addition, the number of spontaneously active neurones encountered in each animal and the mean spontaneous firing rates of these neurones did not differ significantly in drug treated and saline treated groups.

A speculative interpretation of these results could be as follows: It is known that both excitatory and inhibitory receptors for ACh are present in the cortex, and it is possible that both types co-exist on the same neurones. Atropine, imipramine and viloxazine at the doses used may preferentially block the inhibitory receptors. The persistent blockade which would occur during chronic administration could result in an increased sensitivity or number of the inhibitory receptors. This would conceivably manifest itself as an increase in the number of cells depressed by iontophoretically applied ACh. Whatever the explanation it seems that antidepressants as well as causing quantitative changes in the sensitivity of monoamine systems may also result in a qualitative change in sensitivity of cholinergic systems in the long term.

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ACTION OF CHRONIC HIGH LEVEL OF ENDOGENOUS NEUROPEPTIDES IN RATS

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It is difficult to study the behavioral action of chronic high levels of endogenous neuropeptides, because the repeated injection of a drug for long periods of time does not reproduce the condition of continuous high

levels of neuropeptides. We have reported that rats bearing the MtT-F4 tumor developed high level of blood beta-MSH, beta-LPH and beta-endorphin-like substances, thus providing a suitable preparation. Our objective is to study this unique preparation. In this abstract we report a study on the analgesia and gross behavior.

Forty inbred Fisher 344 male rats were used in two series of experiments, one using rats implanted with the pituitary mammotropic transplantable tumor MtT-F4 and other with the mammary gland transplantable tumor R3230AC. The following groups were tested: 1) MtT-F4 A: ten control rats sham implanted. 2) MtT-F4 B: five rats implanted with tumor cells which did not develop tumor at the end of the experiment (41 days). 3) MtT-F4 C: five rats implanted with tumor cells, which developed tumor in 15 days. 4) R3230AC A: ten sham operated rats used as control. 5) R3230AC B: ten rats implanted with tumor cells; all of which developed a tumor during the time of the experiment.

Analgesia was tested twice a week during 5 weeks using a hot-plate heated to $46 \pm 0.5°$ C, during 9 min, in 3 periods separated by 4 - 5 min of rest. The response (paw-licks) was visually controlled. Beta-MSH radioimmunoassay dosage was done at days 26 and 32. Naloxone (0.4 mg/rat) was injected i/p at days 40 and 41.

Results showed significant analgesia ($P < 0.001$) in the group MtT-F4 C which developed tumor; it was associated with a significant increase of blood beta-MSH-like peptide. These rats also showed rigidity, increased docility, diminution of exploration and leanness, which did not appear in other series. Naloxone blocked analgesia and rigidity. Rats of R3230AC groups did not show significant differences between control and tumor-bearing groups. Blood beta-MSH was similar and naloxone did not induce significant changes.

Animals with tumor produced by MtT-F4 cells developed analgesia and some behavioral signs, while rats with tumor produced by R3230AC cells and control rats did not. MtT-F4 cells secrete several peptides: growth hormone, prolactin, ACTH, beta-LPH, beta-MSH and beta-endorphin. Because of the analgesic action of endorphin, the behavioral action of endorphin and ACTH and the lipolytic action of beta-LPH and beta-MSH we propose that rats bearing the MtT-F4 tumor showed a polyneuropeptide syndrome characterized, at least, by analgesia, docility, rigidity, diminution of exploration and leanness.

AIRWAY CONSTRICTION FOLLOWING STIMULATION OF THE GLOSSOPHARYNGEAL NERVES

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Stimulation of chemoreceptors in the carotid body or baroreceptors in the carotid sinus has been reported to produce reflex constriction of the airways. The afferent pathways of these reflexes involve the glossopharyngeal nerves (IXn); efferent pathways are not well defined. We have shown previously that diazoxide and serotonin produce reflex airway constriction, and have suggested that, in the guinea pig, this constriction results from the stimulation of distinct reflex pathways involving the baroreceptors and chemoreceptors, respectively. We have therefore investigated the effects of orthodromic stimulation of the carotid sinus nerve on dynamic pulmonary compliance (C_L) and flow resistance (R_L) in the anesthetized, paralyzed guinea-pig.

The procedures for measuring C_L and R_L have been described previously (Biggs and Peterson, Agents and Actions, in press and Proc. West Pharmacol. Soc., 22, 257, 1979). The right IXn was exposed as it emerged from the jugular foramen. The carotid branch was cleared from any connective tissue and severed. The free central end of the nerve was drawn into a suction electrode filled with 0.9% saline and stimulated with square wave pulses (pulse width, 0.0125 - 3.2 msec, frequency, 5 Hz) at either 2 or 4 V for periods of 5 sec using a Grass S44 stimulator.

Nerve stimulation (NS) at 2 or 4 V produced no observable changes in C_L but caused increases in R_L which were dependent on pulse width. Threshold responses were obtained near a pulse width of 0.0125 msec and maximum responses were obtained near 0.2 msec in most animals. Bilateral, cervical vagotomy had no significant effect on these responses.

Atropine (ATR), 0.05 mg/kg, mepyramine (MEP), 0.1 mg/kg, or disodium cromoglycate (DSCG), 10 mg/kg, all reduced but failed to completely block responses to NS. Additional doses of these drugs failed to abolish responses to NS. However, combinations of ATR and MEP or ATR and DSCG could completely eliminate the increases in R_L produced by NS. Combinations of MEP and DSCG were no more effective than either drug used alone.

We have concluded from these results that stimulation of afferent fibers in the IXn involves two distinct mechanisms which are independent of the vagus nerves. One of these involves a cholinergic pathway which can be blocked with ATR, whereas the other appears to involve H_1 receptors and can be blocked by either MEP or DSCG. These results are in agreement with our previous work which suggests two separate pathways for reflex constriction due to the stimulation of either chemoreceptors or baroreceptors.

ANESTHETICS CAN SELECTIVELY DISRUPT THE NORMAL ACTIVITY OF A SINGLE ISOLATED NEURON

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It is generally accepted that the only requirement for activity of anesthetic agents is lipid solubility, therefore these agents are commonly classified as non-specific drugs. The interaction of these agents with neuronal membranes is apparently dependent only on the physicochemical properties of the drug, the ability to affect excitable cells is not related to molecular structure. These observations have provided the support of a unitary theory of action of anesthetics, i.e. all anesthetics act in a similar manner. However, a unitary theory of mechanism cannot account for the differences in action observed in whole animal or at the cellular level.

Anesthetics can display a spectrum of effects ranging from excitation to depression.The phenomenon of anesthesia may be associated with both functional activation and/or depression of neuronal activity. In addition to a requirement of lipid solubility, specificity may occur at the cellular or membrane level.

In order to test this hypothesis, several anesthetics were studied on the firing output of a single isolated neuronal cell, the abdominal stretch receptor of the crayfish (Procambarus clarkii). The results provide support that anesthetics can selectively alter neuronal activity which suggests that more than one mechanism may be involved in anesthesia.

The profiles of activity of alcohols, barbiturates, steroid and volatile anesthetics and local anesthetics were not similar. Most agents were capable of exciting and depressing the excitability of the neuron, and these effects were concentration dependent. In addition, some anesthetics, primarily the alcohols and volatile agents produced differential effects on the firing output of the cell at concentrations just below those required for complete depression. Some anesthetics were capable of disrupting the normal rhythmical output of the stimulated receptor cell. The drug-induced arrhythmical pattern of output consisted of bursts which were different for various agents.

Using intracellular techniques, the effects of three selected anesthetics were studied on the membrane resistance, resting membrane potential, threshold firing voltage and GABA-mediated synaptic activity. Ethanol, halothane and pentobarbital do not produce similar effects as measured by these parameters which suggest alteration of neuronal activity may be mediated via different mechanisms.

It is postulated that the interaction of an anesthetic with a single neuronal cell may be selective dependent on molecular structure in addition to lipid solubility.

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SERUM PROLACTIN AND GROWTH HORMONE LEVELS IN CHRONIC NEUROLEPTIC RESPONSIVE AND NEUROLEPTIC NONRESPONSIVE MALE SCHIZOPHRENICS

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The dopamine (DA) hypothesis has proven to be valuable in organizing neuroendocrine research in schizophrenia. Current views suggest that in schizophrenia the DA malfunction is more likely to be a type of receptor supersensitivity rather than an exaggerated DA release. Dopaminergic

activation leads to a suppression of prolactin and an increase of growth hormone. Thus, if the postulated DA hypersensitivity is generalized, prolactin responses may help differentiate neuroleptic responders from nonresponders, the latter would be expected to show attenuated prolactin and growth hormone responses.

The objective of this study is to determine whether baseline prolactin and growth hormone values differentiate chronic neuroleptic-responsive schizophrenic out-patients from similarly chronic inpatients whose disorder has not been sufficiently controlled to allow their discharge from hospital after many years. Only male patients aged 18 to 40 years, free from major medical illness, and having a certain diagnosis of schizophrenia (Research Diagnostic Criteria — Spitzer et al) were selected.

Single 8:00 a.m. blood samples were taken on the two days prior to and on the second day following fluphenazine decanoate injection (fzd) for all patients receiving this treatment. The total daily dose of all neuroleptics was fixed for the duration of the study and was transformed into chlorpromazine (CPZ) equivalents based on J.N. Davis (1976). The inpatients were receiving a mean daily dose of 2049 mg. CPZ equivalents while the corresponding out-patient value was 382 mg.

Growth hormone was assayed by the method of Schalch et al (1964) and prolactin according to the method described by Huang et al (1971).

Virtually all patients showed complete suppression of growth hormone,all values below 2 ng./ml.; and this data was not analyzed further.

Although a prolactin rise might be expected 48 hours after fzd,analysis of variance showed this was not the case for either group of patients. However, when the 3 day mean prolactin values were examined it was found that the inpatient mean value of 33.3 ng./ml. was significantly greater than that of the outpatients at 18.1 ng./ml.

Analyzing data from all subjects revealed a significant linear correlation ($r=0.46$, $p<0.05$) between mean serum prolactin and daily CPZ equivalent neuroleptic dose.

Thus, the relatively responsive schizophrenic outpatients were characterized by both lower neuroleptic doses and correspondingly lower serum prolactin values. These results coupled with recent observations indicating that clozapine, a potent neuroleptic, has little influence on serum prolactin both suggest that the DA mechanisms reflected in the serum prolactin are not likely to be the best system for selecting future neuroleptics nor for predicting the clinical response in individual patients.

EFFECTS OF VESTIBULAR ACTIVATION ON SMOOTH PURSUIT TRACKING PERFORMANCE IN PSYCHIATRIC PATIENTS

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The present investigation examining possible vestibular involvement in impaired smooth pursuit tracking in psychiatric patients was prompted by: (a) the history of suggested abnormal vestibular functioning in schizophrenia; (b) known vestibular involvement in smooth pursuit and saccadic eye movement systems; and, (c) the recent demonstration that patients with central vestibular disorders show significantly less suppression of calorically induced nystagmus during smooth pursuit tracking than patients with peripheral vestibular disorders or normals.

Thirty psychiatric patients (15 inpatients — IP: psychotics, requiring intense psychiatric care; 15 outpatients — OP: psychotics in remission) and 15 non-hospitalized controls (C: no history of psychiatric illness) were subjects. All gave informed consent and were free from organic disorders and medication known to affect vestibular or oculomotor systems or tracking performance. Otoscopic examinations ensured that external auditory canals were unobstructed and tympanic membranes were normal and intact.

Bipolar horizontal and vertical EOG activity, and facial EMG activity were recorded before (baseline) and during serial bilateral caloric irrigation (250 ml; 30°C water) while subjects tracked a target light oscillating at .45 Hz for 30 seconds. Subjects responded (button-press) to random 200 msec. target light interruptions as a monitor of attentiveness. Tracking trials were randomized across subjects. Horizontal EOG recordings were differentiated to obtain the first derivative (eye velocity) and deviant tracking — instances of eye velocity slowing to $\leq 2°$/sec. (velocity arrests: VAs) determined from these tracings. VAs coinciding with head movement or blinking were excluded from analysis. Two individuals scored coded differentiated playbacks with 95% agreement and discrepancies were resolved by consensus. Data were analyzed using repeated measures analyses of variance with post-hoc tests where warranted.

Baseline tracking of controls was superior to that of patient groups ($C<OP$, $p<.05$; $C<IP$, $p<.01$). OP mean tracking scores, intermediate between C and IP group scores, did not differ significantly from the latter. Vestibular activation increased tracking errors and variability, but did not effect significant within-group enhancement of tracking errors. Significant order effects were not found, but tracking during the second activation procedure was associated with group differences not evident after the first procedure, i.e., IP tracking impairment was significantly enhanced relative to that of both OP ($p<.001$) and C ($p<.002$) groups. OP — C baseline differences were not maintained during vestibular activation procedures.

Although the influence of factors other than activation of central vestibular mechanisms (e.g., heightened anxiety or distractibility) cannot be dismissed, the present results suggest that serial activation of the vestibular system selectively enhances tracking dysfunction in actively ill psychotics. (Supported by the Ontario Mental Health Foundation)

EXTRAPYRAMIDAL SYMPTOM RATING SCALE

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The Extrapyramidal Symptom Rating Scale (ESRS) was developed during a study of tardive dyskinesia in 261 schizophrenic outpatients on long-term neuroleptic treatment. It consists of a questionnaire of parkinsonian symptoms, a physician's examination of parkinsonism and dyskinetic movements and a clinical global impression of tardive dyskinesia. The parkinsonism questionnaire, which includes 9 items (restlessness, nervousness, inability to keep still; impression of slowness or weakness; difficulty walking or with balance; difficulty swallowing or talking; stiffness; cramps or pains in limbs, back or neck; tremors, shaking; oculogyric crisis or dystonic reactions; increased salivation) allows patients to report extrapyramidal symptoms experienced at periods other than the time of their examination. The physician's examination for parkinsonism includes 8 items (expressive automatic movements (facial mask/speech); bradykinesia; rigidity (limbs); gait and posture; tremor (limbs, head, chin and tongue); akathisia; increased salivation; dystonia). Tardive dyskinesia is rated following a standard procedure designed to activate or uncover covert dyskinesias. Dyskinetic movements in each of the commonly-involved muscle groups (tongue, jaw, lips, trunk and extremities) are rated according to their frequency and amplitude.

Validity — The scale was validated in 8 double-blind studies: 2 comparing the extrapyramidal effects of different neuroleptics in the treatment of acute and chronic schizophrenia, 2 assessing the effects of neuroleptic withdrawal on the evolution of extrapyramidal symptoms, 3 comparing different kinds of antiparkinsonian agents in the treatment of drug-induced extrapyramidal symptoms and one comparing deanol with placebo in the treatment of tardive dyskinesia. The ESRS was found to be sensitive in its ability to detect changes in both parkinsonian and dyskinetic symptoms and to give results consistent with those from studies using standard scales such as the Involuntary Abnormal Movement Scale. The ESRS ratings were also shown to correlate significantly with biological measures of drug action such as prolactin levels.

Reliability — A reliability study was carried out in which a neurologist and 2 psychiatrists independently rated 89 schizophrenic outpatients on long-term neuroleptic treatment. Inter-rater reliability coefficients were calculated for each item of the scale, and ranged from 0.80 to 0.97. The lowest coefficients were for expressive automatic movements (0.80) and abnormal truncal movements (0.82).

These results show the ESRS to be a highly sensitive and reliable instrument for measuring parkinsonian and dyskinetic symptoms.

GABAERGIC MECHANISMS AND ALCOHOLISM

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The wealth of recent evidence implicating GABAergic processes in the etiology of alcoholism is reviewed and synthesized into a "GABA

hypothesis for alcoholism". The cornerstones of this hypothesis are the following. 1) Anxiety plays a role in the etiology of alcoholism. 2) GABA occupies a strategic position in the neurophysiology of anxiety. 3) Acute ethanol (ethyl alcohol) administration alleviates anxiety by potentiating GABAergic neurotransmission. 4) Chronic ethanol intake decreases GABA levels and alters the density of GABA receptors, initiating a vicious cycle, since higher amounts of ethanol are needed to produce the anxiolytic effect or to avoid the withdrawal symptoms.

Anxiety is implicated in the etiology of alcoholism by a wealth of data in the literature. 1) Ethanol's anxiolytic properties have been demonstrated experimentally, both in animals and man. 2) The psychological profile of alcoholics is characterized by high anxiety. 3) A significant percentage of alcoholics report psychological distress and family or employment problems as the reason for drinking. 4) Finally, voluntary ethanol consumption is increased under conditions of experimental stress.

The central role of GABA in the neurophysiology of anxiety is suggested by the fact that both benzodiazepines and barbiturates, which are the most commonly prescribed anxiolytic drugs, are known to enhance GABAergic neurotransmission. Furthermore, the relative affinities of pharmacologically active benzodiazepines for the benzodiazepine receptor correlate well with their ability to antagonize GABA-modulin (the endogenous inhibitor of GABA receptors) *in vitro*, as well as with their GABA-potentiating potencies *in vivo*. Moreover, the specific effects of anxiolytic benzodiazepines in animal experiments involving a conflict between reward and punishment are mimicked by muscimol (a GABA agonist) and are eliminated by bicuculline (a specific GABA antagonist) and by thiosemicarbazide (an agent decreasing GABA synthesis).

As for the interaction of ethanol with GABAergic processes, ethanol potentiates GABA-mediated spinal presynaptic inhibition. Furthermore, in the cerebral cortex, ethanol potentiates GABA-mediated inhibition of single neurons, but not inhibition induced by glycine, dopamine or serotonin. Moreover, bicuculline (a specific GABA antagonist) diminishes the behavioral manifestations of ethanol intoxication, whereas aminooxyacetic acid (AOAA) (an inhibitor of GABA catabolism) markedly decreases these behavioral manifestations. Lastly, chronic ethanol intake, in contrast to acute ethanol administration, decreases brain GABA levels, and decreases the density of low-affinity GABA receptor sites.

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A PROPOSAL FOR THE PATHOGENESIS OF PARKINSON'S DISEASE BASED ON DOPAMINE NEUROTOXICITY

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Since its first description by Couper in 1837, manganese (Mn^{++}) neurointoxication has intrigued researchers. There is a remarkable similarity between manganism and the neuropathology, chemistry and clinical symptomatology of Parkinson's disease. At autopsy, brains in both cases exhibit depigmentation of the melanin granules along with neuronal degeneration of the substantia nigra and, to a lesser extent, the locus coeruleus. Also, both types of disorder are typified by dopamine (DA) deficiency in the caudate-putamen and norepinephrine (NE) in the hypothalamus. We have noted a remarkable similarity in the neurochemical and behavioral effects of Mn^{++} or 6-hydroxydopamine (6-OHDA). Intracerebroventricular (ICV) injection of Mn^{++} or 6-OHDA elicits rotational behavior in conscious rats, while bilateral intrastriatal injections produce a "freezing" or reserpine-like syndrome. Using similar routes of injection both agents can deplete NE in the hypothalamus without affecting DA. Such similarities in effects of Mn^{++} and 6-OHDA could be due to (a) the ability of Mn^{++} to promote 6-OHDA synthesis in nervous tissue, or, (b) to another mechanism. Since 6-OHDA cytotoxicity arises from its autoxidation byproducts, O_2^-, H_2O_2, and $\cdot HO$, Mn^{++} may act similarly. Autoxidation of DA can result also in formation of free-radicals and orthoquinone which are toxic to neuroblastoma cells (Graham et al, Mol. Pharmacol. 14, 644, 1978). We found Mn^{++} to markedly potentiate DA autoxidation in comparison to Cu^{++}, Zn^{++} Ni^{++}, Ca^{++} or Mg^{++}. The Mn^{++}-enhanced autoxidation of DA was accompanied by generation of O_2^-, H_2O_2, and $\cdot HO$ as suggested by the inhibitory effects of superoxide dismutase, catalase and ethanol.

Free radicals may exert their toxicity per se by inhibition of the sulfhydryl moieties present on biomembranes. In the brain, the enzymes particularly sensitive to O_2^- are glutamic acid decarboxylase and Na-K-ATP'ase both of which play a critical role in neurotransmission. Free radicals can initiate lipid peroxidation by attacking polyunsaturated fatty acids on neuronal membranes. The basal ganglia represents the ideal milieu for lipid peroxidation due to its unique DA, metal ion, and unsaturated fatty acid content which with high oxidative activity render this brain region especially vulnerable to lipid-peroxide mediated neurodegeneration.

The principal substrate for neuromelanin in the substantia nigra is DA. DA autoxidation leads to free radical formation as well as quinones, both agents which are toxic to cells, thus over the course of time autoxidation could produce progressive cell injury and ultimately neuronal death. In Parkinson's patients the use of l-dopa to enhance dopamine levels in the neostriatum could be expected to accelerate neurodegeneration while temporarily alleviating some of the extrapyramidal symptoms.

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CHLORDIAZEPOXIDE NORMALIZES ABNORMAL BEHAVIOR AND HORMONE RESPONSES TO STRESS IN SEPTAL RATS

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When lesioned in the septal nuclei rats display a dramatic change in behavior in response to stress, characterized by increased responsiveness to environmental disturbances. Associated with the behavior disturbance is a parallel disturbance in the pituitary-adrenal axis characterized by increased corticosterone responsiveness in response to stress. Chlordiazepoxide, a benzodiazepine, is known to reduce the behavioral hyperresponsiveness of septal rats and the present study was undertaken to see if it would also normalize the adrenal hyperresponsiveness. Three days after surgery, when behavior and adrenal disturbances are maximal following septal lesions, various groups of normal sham operated and septal lesion rats were injected with chlordiazepoxide (15 mg/kg i.p.), saline or nothing at a given time of day. Two hours later they were behavior rated on several items including resistance to capture, magnitude of first startle response and number of consecutive startles, each item having a maximum rating of 4. Blood was collected and assayed for corticosterone and brains were analyzed for extent of lesion. Septal rats displayed the expected behavior and adrenal hyperreactivity in response to the stress of behavior rating in comparison with non-lesioned controls. Septal rats which received chlordiazepoxide exhibited normalized behavior and corticosterone scores both in comparison with other septal groups and non-lesioned control groups. It is suggested that the behavior and hormone disturbances seen following septal lesions may be mediated by the transmitter GABA and that chlordiazepoxide is a useful drug for reducing excessive behavioral and adrenal responsiveness to stress.

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REGIONAL DISTRIBUTIONS OF *m*- AND *p*-HYDROXYPHENYLACETIC ACIDS IN RAT BRAIN

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The endogenous concentrations of *m*- and *p*-hydroxyphenylacetic acids, the acid metabolites of the trace amines *m*- and *p*-tyramine, have been determined in five rat brain regions using deuterated internal standards and capillary column-high resolution GC-MS. The tissues were homogenized in $ZnSO_4$ solution, to which had been added 50 ng each of pentadeutero-m-hydroxyphenylacetic acid and tetra-deutero-p-hydroxyphenylacetic acid. After precipitation of the protein with $Ba(OH)_2$ and centrifugation at 1000 x g for 10 min. the acid fraction was extracted from the acidified supernatent into ethyl acetate and derivatized first with methanolic-HCl and then heptafluorobutyric anhydride. The methylheptafluorobutyryl di-esters of the *m*- and *p*-acids were separated on a 57m SP2250 SCOT capillary column and quantitated from the integrated ion current profiles of their molecular ions at m/e 362.0389, m/e 366.0640 and m/e 367.0703.

Concentrations of *m*-hydroxyphenylacetic acid in the regions (mean ±

SEM, and number of measurements in brackets) were: caudate nucleus, 5.5 ± 0.6 (5); hypothalamus, 1.2 ± 0.3 (5); brain stem, 1.8 ± 0.1 (5); cerebellum, 1.2 ± 0.1; and the rest 1.7 ± 0.1 (5) nanograms/gram. Concentrations of *p*-hydroxyphenylacetic acid in the regions were: caudate nucleus, 28.3 ± 1.5 (5); hypothalamus, 4.5 ± 0.1 (4); brain stem, 8.6 ± 0.6 (5); cerebellum, 8.1 ± 0.4 (5) and the rest 5.3 ± 0.5 (5) nanograms/gram. The whole brain values for the *m*- and *p*-isomers were 2.3 ± 0.3 (7) and 10.6 ± 0.7 (7) ng/g.

From the m/p ratios it was found that the para-isomer concentration was slightly increased in the caudate nucleus, brain stem and cerebellum and decreased with respect to the meta in the 'rest'. The heterogenous distribution of both acids occurring throughout the regions was analogous to those obtaining for the precursor amines.

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A NOVEL PROCEDURE FOR ISOLATION AND QUANTIFICATION OF PARA-TYRAMINE IN RAT BRAIN

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The phenolic amine para-tyramine (*p*-TA) is a normal constituent of brain, and concentrations of this amine have been shown to be increased markedly by monoamine oxidase (MAO) inhibitors and decreased by reserpine and acute doses of several antipsychotics. Because of the amphoteric nature of this substance, it is difficult to extract it from aqueous solutions. This can be overcome by acetylation in the aqueous phase followed by extraction and subsequent perfluoroacylation of the N-acetylated portion of the molecule. However, this results in formation of a derivative with a long retention time, and there is interference from other substances present in brain. The assay has therefore been modified by the inclusion of a step, following the acetylation, to specifically hydrolyze the acetylated phenolic function and free it for perfluoroacylation.

Male Sprague-Dawley rats were sacrificed by cervical fracture and the brains were rapidly removed and put in glass vials. Samples were stored at -60° until the time of analysis. The brains were homogenized in cold 0.4 N perchloric acid, centrifuged at 10,000 x g for 20 min, and the resultant supernatant was adjusted to pH 7.8. This was followed by extraction into di(2-ethylhexyl)phosphoric acid (DEHPA), 2.5% in chloroform, and back extraction into 0.5 N HCl. The HCl phase was neutralized with solid sodium bicarbonate, and the samples were acetylated as described by Martin and Baker (Biochem. Pharmac., 26, 1513 (1977). The solutions were extracted with ethyl acetate and the phenolic functions hydrolyzed by shaking with a small volume of ammonium hydroxide solution. The ethyl acetate phase was taken to dryness under a stream of nitrogen, and the residue was reacted with trifluoroacetic anhydride. The reaction mixture was partitioned between cyclohexane and saturated sodium tetraborate buffer (Martin and Baker, 1977). For electron-capture gas chromatographic analysis, an aliquot (1.0 µl) of the cyclohexane phase was injected onto a 10 m WCOT (SP-2100) glass capillary column.

There is complete separation of *p*-TA from its *o*- and *m*-isomers, and the method provides adequate sensitivity for quantification of *p*-TA in control whole rat brain. The mean value (± SEM) obtained is 1.8 ± 0.2 (ng/g wet brain, n=5). In rats treated chronically with the MAO inhibitor tranylcypromine (10 mg/kg daily for 7 days; rats sacrificed 6 hours after the last dose), this value increased to 18.7 ± 2.8 (n=5).

A number of other amines, including 2-phenylethylamine, phenylethanolamine, tryptamine, 5-hydroxytryptamine, noradrenaline, dopamine, normetanephrine, 3-methoxytyramine, metanephrine, *m*- and *p*-octopamine and synephrine were tested and shown not to interfere with the assay. Substances which proved to be suitable internal standards for the assay included benzylamine, 3-phenylpropylamine and 2-(4-chlorophenyl) ethylamine.

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RADIOIMMUNOASSAY FOR FLUPHENAZINE IN HUMAN PLASMA

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Fluphenazine is widely used for the treatment of schizophrenia and paranoid states. It is available for oral administration as a dihydrochloride salt, whereas for prolonged effects, its decanoate or enanthate esters are given as intramuscular injectables in sesame oil. The study of correlation of plasma levels of this drug with clinical efficacy is complicated by the fact that fluphenazine is administered to patients in small doses and the slow release of the drug from depot preparations results in plasma concentrations which are much lower than chlorpromazine. Other contributing factors which are responsible for low plasma concentrations of fluphenazine are, an extensive metabolism and a very large 'first pass effect'.

In order to study the pharmacokinetics and correlation of plasma levels of fluphenazine with clinical effect, a sensitive, specific and rapid radioimmunoassay procedure was developed. The assay based on an antiserum to a bovine serum albumin conjugate of 0-(3-Carboxypropionyl) -fluphenazine enables the quantitation of 50 pg of the drug in 200 µL of plasma with negligible cross-reactivity with most of its metabolites. The method is suitable for both single dose pharmacokinetic and therapeutic monitoring in patients following intramuscular treatment with the long acting depot preparations of fluphenazine decanoate or enanthate.

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REDUCTION OF PLASMA LEVELS OF FLUPHENAZINE AND PERPHENAZINE BY RUBBER STOPPERS USED IN VACUTAINERS

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Several studies in the recent literature indicate that the plasma concentrations and the protein binding properties of several basic lipophilic drugs are altered by blood collection procedures involving the use of Vacutainers (Becton-Dickinson). Plasma concentrations of psychotropic drugs such as imipramine, amitriptyline, and chlorpromazine have been observed to be dramatically reduced when blood was shaken, or mixed by gentle inversion, in rubber-stoppered Vacutainers. It has also been shown that plasma concentrations of chlorpromazine, imipramine and amitriptyline were always lower in samples collected with rubber-stoppered Vacutainers than those collected in a similar manner but without allowing the blood to contact rubber stoppers. This reduction in plasma concentration of psychotropic agents has been attributed to a chemical, tri(2-butoxyethyl)phosphate, which is coated on rubber stoppers used in Vacutainers. This chemical which leaches out of the rubber stoppers alters the distribution of psychotropic agents between plasma and whole blood.

The effect of rubber stoppers in Vacutainers on the concentration of fluphenazine and perphenazine in plasma was investigated. Blood was collected in Vacutainers from dosed healthy volunteers and patients undergoing therapy with the drug. A technique was used which kept the blood from coming into contact with the rubber stopper. At the same time a blood sample was obtained in each case, in the conventional manner, which allowed the blood to come into contact with the rubber stoppers. Plasma samples were analysed for fluphenazine and perphenazine by specific and sensitive radioimmunoassay procedures. It was noted that the fluphenazine and perphenazine concentrations in plasma samples from blood which were in contact with the rubber stoppers were always lower than those samples where the blood did not touch the rubber stoppers. Thus the use of Vacutainers for the collection of blood samples for quantitative analysis of these psychotropic agents should be avoided, unless extreme care is taken to keep the blood from coming in contact with the rubber stoppers.

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HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF CHLORPROMAZINE AND ITS METABOLITES IN PLASMA

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A quantitative high pressure liquid chromatographic (HPLC) method for the determination in plasma of chlorpromazine (CPZ) and the metabolites chlorpromazine sulfoxide (CPZSO), 7-hydroxychlorpromazine (7-OHCPZ), desmethylchlorpromazine (Nor_1CPZ), didesmethylchlor-

promazine (Nor_2CPZ) and chlorpromazine N-oxide (CPZNO) has been developed.

Extraction of CPZ and its metabolites is achieved from alkalinized plasma with ethyl ether containing 5 percent n-butanol. The ether-n-butanol is extracted into 0.05N HCl which is then made alkaline with 1N ammonia and again extracted with ether-n-butanol. The ether-n-butanol extract is evaporated to dryness under nitrogen and the residue dissolved in 100 μl of methanol. Aliquots are injected into a 30 cm x 3.9 mm i.d. column packed with 10 μm average diameter silica particles containing a chemically bonded octadecyl monolayer (μ Bondapak C_{18}, Waters Associates). The mobile phase consists of a 65:35 mixture of methanol: aqueous 0.05M Na_2HPO_4 which contains 1 percent acetic acid and 0.005M 1-pentane sulfonic acid, Na salt. This mixture is titrated to an apparent pH 6.0 with 1N NaOH. A fixed wavelength detector, 254 nm, (Model 440, Waters Associates) and in series an electrochemical detector with a glassy carbon electrode set at +0.9 V vs the Ag/AgCl reference (TL-4 thin-layer detector cell, Bioanalytical Systems) are used. Standard curves are prepared by adding CPZ and its metabolites to drug free plasma. The method is applicable to therapeutic concentrations of CPZ and its metabolites.

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STUDIES ON THE ISOLATION AND QUANTITATION OF HYDROXYLATED AMINES IN AQUEOUS SOLUTION

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The analysis of drugs and their metabolites in urine presents a number of difficulties. The procedure adopted must be capable of extracting these compounds efficiently, of separating them from other components present in the urine, of unequivocally identifying the separated compounds and of quantitating them by methods which prevent or minimize decomposition and are specific and sensitive. Novel methods of analyzing hydroxylated amine drugs and metabolites with CNS activity (e.g. phenylephrine, p-tyramine, p-octopamine, p-hydroxyamphetamine, ephedrine, norephedrine, morphine, codeine and hydroxylated aniline isomers) in aqueous solution have been investigated. Two extraction procedures were evaluated. Each drug was acylated in aqueous solution with acetic anhydride (Martin and Baker, Biochem. Pharmacol., 26, 1513, 1977) or mono- or dichloroacetic anhydride and then extracted into an organic solvent. Alternatively, a phase-transfer extraction procedure was used in which a small volume of acetonitrile was added to the aqueous solution prior to extraction (Singh et al, Bull. Environm. Contam. Toxicol., 23, 470, 1979). Using either extraction procedure resulted in good to excellent partitioning of the acylated drug or metabolite into the organic solvent. In some instances, the concentrated organic solution was suitable for analysis without further treatment. In other instances, the extracted acylated compound was further reacted with trifluoroacetic anhydride or another perfluoroacylating reagent prior to analysis by gas chromatographic (GC) and mass spectrometric (MS) analysis. All derivatives were separated and unequivocally identified by combined GC/MS. The quantitative analysis of the derivatized compounds was performed on packed columns in a gas chromatograph equipped with a flame ionization detector or on a capillary column in a gas chromatograph equipped with an electron-capture detector. The sensitivity of the assay procedure varied with different compounds but in most instances low nanogram quantities were readily analyzed quantitatively.

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KINETICS OF MATERNAL-FETAL ALCOHOL EXCHANGE

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The Fetal Alcohol Syndrome (FAS), first described as a clinical entity in 1973, was suggested by Jones et al. (Lancet, 1, 1267, 1973) to be the third leading cause of mental retardation in the north-western United States. While many studies of alcohol kinetics have been carried out in nonpregnant humans, determination of kinetic parameters in pregnant subjects has not been studied. Accordingly, the present series of experiments was initiated to investigate maternal-fetal alcohol exchange using the pregnant ovine model.

Early in gestation (0.75 gestation) arterial, venous and amniotic fluid cannulae were implanted in the fetus and arterial and venous cannulae in the ewe. All experiments were carried out at approximately 119 days of gestation (0.81 gestation). Dilute ethanol (20%) was infused over a period of 30 min through the maternal venous cannula. Samples of whole arterial blood (maternal and fetal) and amniotic fluid were withdrawn at various times after the start of the infusion. Alcohol concentrations were determined by a gas chromatographic method using n-propanol as an internal standard.

A three-compartment model can be used to describe the data with elimination from the maternal compartment following Michaelis-Menten kinetics. Following infusion into the maternal circulation, peak concentrations in the maternal and fetal compartments were very similar. In addition, the rate of transfer of alcohol from the mother to the fetus was very rapid; alcohol could first be detected in fetal blood at 2-4 min after the start of infusion and the peak fetal blood alcohol level occurred at 12-15 min after the end of infusion. The rate of rise of fetal blood alcohol was 4.6-5.5 mg ml^{-1} h^{-1}.

In contrast to the maternal-fetal exchange, equilibration between the maternal and amniotic fluid compartments through the intervening fetal compartment proceeded at a much slower rate. Alcohol was first detected in the amniotic fluid at 2-7 min after the start of the infusion and the peak amniotic fluid level was attained at 1.3-2.8 h after infusion ceased. Peak amniotic fluid concentrations were lower than those attained in fetal blood; maximum amniotic fluid concentration was 70-78% of the maximum fetal blood concentration. Rates of elimination from the three compartments appeared to be similar (0.14-0.23, 0.13-0.19, 0.14-0.19, mg ml^{-1} h^{-1} for maternal, fetal and amniotic fluid respectively).

The data suggest that alcohol reaching the maternal circulation establishes a rapid equilibrium with the fetal circulation; thus any maternal exposure to alcohol would be expected to result in fetal exposure as well. Further research will be necessary to establish the minimum fetal alcohol concentration resulting in the neurological dysfunction which is a characteristic component of FAS.

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CENTRAL NERVOUS SYSTEM STIMULANTS AND THE UPTAKE AND RELEASE OF DOPAMINE AND *p*-TYRAMINE IN RAT STRIATUM *IN VITRO*

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Para-tyramine (*p*-TA) is a phenolic amine whose presence in brain has been confirmed in recent years by several sensitive analytical techniques. Its role in the central nervous system is unclear, but because it is concentrated in the striatum and because of its structural similarity to dopamine (DA), it has been suggested that its activity may be closely linked to this putative neurotransmitter. Although studies on uptake kinetics in striatal slices have shown the Km for *p*-TA to be similar to that for DA, experiments with various inhibitors of amine uptake such as benztropine have suggested that the uptake systems for the two amines are in fact different. This has now been extended to a study of other drugs affecting DA transport, (L. Dyck, personal communication) and we report here the results of some experiments with amphetamine (AMPH) and methylphenidate (MPD) on the uptake and release of ^{3}H-labelled DA and *p*-TA in prisms of rat striatum.

Male Wistar rats were sacrificed and the corpus striata dissected out and placed on an ice-cooled plate. Prisms of tissue were prepared (0.1 mm x 0.1 mm x 2.0 mm) using a McIlwain tissue chopper. Uptake and release were then studied using the method of Raiteri et al. (Eur. J. Pharmacol., 34, 189, 1975). The concentration of DA and *p*-TA used in these experiments was 10.0 nM. All drug concentrations are expressed in terms of the free base. Pargyline (50.0 μM) was present in all incubation and superfusion media.

d-AMPH was stronger than MPD in both inhibiting uptake and stimulating release of DA and *p*-TA. Studies of the drugs on uptake and release of *p*-TA and DA revealed considerable differences on the two processes for each drug. While *d*-AMPH was a slightly stronger inhibitor of *p*-TA uptake than of DA uptake, it was a stronger releaser of DA than of *p*-TA. MPD was virtually equipotent with regard to its effects on DA and *p*-TA uptake, but caused a much greater stimulation of *p*-TA release than of DA release. These results would appear to confirm other reported studies which indicated differences in transport systems for the two amines (Petrali, Neurochem. Res., 5, 297, 1980).

Preliminary studies with *d*- and *l*-AMPH at a concentration of 1.0 μM have revealed that, as with DA, the *d*-isomer is a considerably stronger releaser of *p*-TA than is the *l*-isomer.

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A GAS CHROMATOGRAPH TECHNIQUE USING ELECTRON CAPTURE DETECTION FOR SIMULTANEOUS ESTIMATION OF TRYPTAMINE AND 5-HYDROXYTRYPTAMINE IN BIOLOGICAL TISSUE

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It has been proposed that mood depression may result from the deficit of certain biogenic amines at synaptic sites in the brain. Amines implicated in this regard have included 5-hydroxytryptamine (5HT), noradrenaline, and the "trace" amines tryptamine (T) and 2-phenylethylamine. We have developed a relatively simple and rapid gas chromatography (GC) method for the simultaneous quantification of two of these amines, T and 5HT, in human urine and rat brain.

Very briefly T and 5HT in 1 ml urine or 4-8 ml of a homogenate of rat brain, are extracted into di(2-ethylhexyl)phosphoric acid (2.5% v/v in chloroform), back extracted into 0.5 N HCl, and acetylated using acetic anhydride. The acetylated amines are then extracted into ethyl acetate, which is taken to dryness and reacted with 75 μl pentafluoropropionic anhydride (PFPA) for 30 min at 60°C. Excess PFPA is removed by partitioning between 300 μl cyclohexane and 3 ml saturated sodium tetraborate solution. 1 μl of the cyclohexane is used for GC analysis.

GC conditions are as follows: column: WCOT glass capillary, OV-101, 10 m, 0.24 mm ID; carrier gas: helium, 7 psi; make-up gas at detector, 10% methane in argon, 36 ml/min; oven temperature: 80°C, increasing at 20°C/min to 220°C; injection port and detector temperatures: 250°C; retention times for the derivatives of T and 5HT, 7.3 and 9.5 min respectively. 5-Methyltryptamine is used as the internal standard.

The method has been used to measure T and 5HT in 24 hr urine samples from 15 healthy volunteers. Mean values (± S.E.M.) for T and 5HT are 80.2 ± 16.2 and 140 ± 28 μg/24 hr, respectively. T and 5HT have been quantified in rat whole brain tissue from control rats, from tryptophan (Tp)-treated rats, and from rats treated with the monoamine oxidase inhibitor tranylcypromine (TCP) with and without a Tp load. Drug schedules used were as follows: TCP, 20 mg/kg, 1.5 hr before death; Tp, 100 mg/kg, 1 hr before death; controls, saline injected 1.5 hr before death. Results are presented as means ± S.E.M. in ng/g. N=6.

Treatment	T	5HT
Control	< 1	581 ± 27
Tp	< 1	700 ± 26
TCP	49.0 ± 2.5	714 ± 21
TCP + Tp	142 ± 11	1427 ± 100

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RADIOIMMUNOASSAY FOR TRIFLUOPERAZINE IN HUMAN PLASMA

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Trifluoperazine, a piperazinyl-linked phenothiazine, is widely used for the treatment of schizophrenics with hallucinatory syndromes, delusions, and disturbances of thinking. It has been recommended for acute phychoses and maintenance therapy for hebephrenia and for chronically hospitalized patients. There are very few studies reported where blood levels of trifluoperazine have been quantitated in either healthy human volunteers following single doses or patients under acute or chronic treatment with this drug. This is due in no small way to the lack of availability of suitable, sensitive and specific procedures for the analysis of this drug in plasma. The study of the pharmacokinetics and the correlation of the plasma levels of trifluoperazine with clinical efficacy in patients is desirable. In order to achieve these objectives, a sensitive, specific and rapid radioimmunoassay (RIA) procedure has been developed. The RIA procedure enables the quantitation of 50 pg of the drug in 200 μl of plasma with negligible cross-reactivity with most of its available metabolites. The method is suitable for both single dose pharmacokinetic study and therapeutic monitoring in patients being treated with the drug.

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THE MULTIPLICITY OF MONOAMINE OXIDASE: MOLECULAR ASPECTS

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Monoamine oxidase (MAO, EC 1.4.3.4.) is a mitochondrial outer membrane enzyme which deaminates amine neurotransmitters in the central nervous system as well as other biogenic amines. At present MAO has been grouped into two types based on substrate specificity and inhibitor susceptibility. From the clinical point of view the multiplicity concept of MAO has become quite important. Our knowledge of the multiplicity phenomenon, however, is still confusing and controversial so that a detailed analysis of the type A and B enzymes remains important. A basic question yet to be answered is whether the different types of MAO activity are due to the existence of different apoenzymes or the effects of other components, such as binding lipids or other membrane substances.

In order to study the possible involvement of membrane phospholipids, amine oxidation by rat liver mitochondrial MAO both before and after lipid-depletion have been compared. The kinetic parameters of the enzyme were significantly changed following the lipid-depletion; no inter-conversion between A and B types was observed.

A study of the molecular structures is difficult, since during solubilization and purification the two types of enzyme change their properties. In this study (^{3}H)-pargyline, a substance that links specifically and irreversibly to the active sites of both A and B MAO, was covalently bound to the active sites of mitochondrial MAO isolated from various tissues. Chosen were rat heart and human placenta to represent type A, pig liver and bovine liver to represent type B, and rat liver and brain which are mixed A and B.

The (^{3}H)-pargyline-MAO adducts were isolated and hydrolyzed by proteolytic enzymes and the labelled peptides (pargyline binding sites) separated and compared by paper chromatographic separation in several different solvent systems and after electrophoresis at various pH values. Only one identical pargyline peptide was obtained from all the different MAO's. The alternative A and B sites were assessed after pre-incubation of rat liver MAO with the selective inhibitors deprenyl (to block the B site) and clorgyline (to block A site). Following proteolysis the (^{3}H)-pargyline peptides of both A and B MAO from the pretreated rat liver mitochondria are also identical.

The pargyline binding peptide of the rat liver MAO has been purified by a series of chromatographic and electrophoretic procedures. Micro-Edmann degradation, followed by dansylation, revealed the amino acid sequence Ser-Gly-Gly-Cys(X)-Tyr.

These findings demonstrate that the immediate surrounding primary structure of the pargyline binding site for both type A and type B MAO are identical; this does not rule out the possibility however that the amino acid sequences of the A and B apoenzymes may be different at some sites remote from the pargyline binding site, since Cawthon and Breakfield recently reported that A and B MAO exhibit different susceptibilities towards proteolysis. This difference was interpreted as being due to divergent amino acid sequences of A and B MAO.

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EFFECT OF NIALAMIDE AND TRANYLCYPROMINE ON RAT WHOLE BRAIN CONCENTRATIONS OF 5-HYDROXYTRYPTAMINE, TRYPTAMINE AND 2-PHENYLETHYLAMINE

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The monoamine oxidase (MAO) inhibitors have been used extensively in the treatment of depression. The rationale for their use is the fact that they inhibit MAO, presumably thereby increasing the amount of monoamine neurotransmitter available at central synapses. However, some drugs which are effective MAO inhibitors are not good antidepressants. Nialamide (NIAL) and tranylcypromine (TCP) have both been reported to be strong inhibitors of MAO (Hendley and Snyder, Nature 220:1330, 1968) but NIAL is less effective as a clinical antidepressant than TCP.

The MAO inhibitors have been shown to cause dramatic increases in the concentrations of the trace amines in brain. Two of these trace amines, tryptamine (T) and 2-phenylethylamine (PE) have been implicated in depression (Dewhurst, Nature 218: 1130, 1968). We report here the effects of NIAL and TCP on the concentration of these two amines as well as the putative amine neurotransmitter 5-hydroxytryptamine (5HT) in rat brain tissue.

Male Wistar rats ranging in weight from 175 to 250 gm were used in this experiment. Animals were sacrificed 1.5 hours after intraperitoneal administration of either 20 mg/kg TCP or 160 mg/kg NIAL. Amines were assayed according to a recently developed gas chromatrography technique with electron capture detection as described in a separate poster at this meeting (Calverley et al.).

Concentrations of the amines (ng/gm wet brain, N=6, mean ± S.E.M.) in whole brain from control rats were: 5HT, 581 ± 27; T, < 1 ng/gm; PE, < 5 ng/gm. After administration of TCP, these concentrations increased to: 5HT, 714 ± 21; T, 49.0 ± 2.5; PE, 42.3 ± 8.1. Whole brains from NIAL treated rats showed the following concentrations: 5HT, 972 ± 48; T, 36.8 ± 1.0; PE, 419 ± 42.

Our results show that at the dosages used here TCP increases T levels to a greater extent than does NIAL but NIAL results in a greater increase in 5HT and PE concentration than does TCP.

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SEASONAL VARIATION IN OCCURRENCE OF POST-PARTUM "BLUES"

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The occurrence and severity of post-partum "blues" was assessed in the early puerperium (days 1-5 post-partum) in a sample of 71 women. These results were obtained as part of a more extensive study of the relationship between biochemical changes and puerperal mood (Handley et al., Br. J. Psychiat., in press). Mood was assessed daily using the depression scale of the Multiple Affect Adjective Checklist (MAACL-D) and a visual analogue scale (VAS). Subjects were scored twice on non-consecutive days during the puerperal period using the Beck Depression Inventory with Pichot addition (BP). Maximum individual scores on the MAACL-D and BP were significantly lower in women giving birth between June and December than between January and April ($p=0.04$ and $p=0.03$ respectively). In addition, the number of "cases" of puerperal blues (women scoring at or above the 80 percentile on at least one scale) was significantly lower in the June-December period ($p=0.01$). Finally, average maximum scores were calculated for each two-month period of the study and were found to peak in January-February, indicating that seasonal factors may play a role in the development of post-partum "blues".

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KYNURENINE EXCRETION IN DEPRESSED STATES

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An increased turnover of tryptophan along the kynurenine pathway has been reported by many authors. Results to date have been conflicting — high, normal and low excretion figures have been reported in depression.

The objective of the present pilot investigation was to test the hypothesis that in depressive illness dietary tryptophan is diverted along the "kynurenine shunt" with the result that 5-hydroxytryptamine levels are decreased and that excess amounts of kynurenine are excreted in the urine.

Twenty-four hour samples of urine from 24 patients diagnosed as suffering from depression (neurotic or psychotic), 5 schizophrenic patients, 2 normal individuals, 2 patients with a personality disorder and 2 patients with organic brain syndrome were analyzed blind for kynurenine. The method of Tompsett (Clin. Chim. Acta, 4, 411, 1959) was used.

In the unipolar and bipolar depressives kynurenine excretion did not differ significantly from excretion in the normals, schizophrenics, personality disorders or brain damaged individuals. Patients diagnosed as suffering from neurotic depressions excreted significantly higher amounts than the other categories ($p < .001$).

It was concluded that the role of stress in the neurotic depressions was important in initiating tryptophan pyrrolase activity and thus diverting tryptophan along the kynurenine pathway.

RELATIONSHIP OF SEROTONIN UPTAKE TO Na^+ and K^+ CONTENT IN DOWN'S SYNDROME AND NORMAL PLATELETS

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The present studies were directed to determine the mechanism of a decreased rate of serotonin (5HT) uptake and decreased 5HT content in Down's syndrome (D.S.) platelets. These platelets have increased Na^+ and decreased K^+ content, a decreased rate of outward movement of Na^+ and decreased inward movement of K^+ and decreased Na^+/K^+ ATPase activity.

	5HT (ng/10^9)	5HT Uptake (nmoles/ hr/10^9)	Na^+ (μg/10^9)	K^+ (μg/10^9)
Control Platelet	3.81	8.89±1.03	3.32±0.21	19.78±0.72
D.S. Platelet	1.50 p<.001	3.65±0.50 p<.001	6.79±0.40 p<.001	13.12±0.48 p<.001

	Na^+/K^+ ATPase (μmoles/ Pi/hr/10^9	Na^+ Out (μmoles/ hr/10^9)	K^+ In (μmoles/ hr/10^9)	
Control Platelet	2.87±0.20	0.903±0.46	0.87±0.05	
D.S. Platelet	2.13±0.19 p<.02	0.441±0.14 p<.001	0.54±0.04 p<.001	

The uptake of 5HT is an electroneutral process that is Na^+ and Cl dependent and linked to the outward movement of K^+. When D.S. platelets are incubated to bring K^+ content to that of normal platelets and 5HT uptake studied, the rates are nearly equal to those of normal platelets. The results suggest that decreased rate of 5HT uptake in D.S. platelets is associated with decreased platelet content of K^+. The results are of interest in view of the proposal that 5HT uptake in platelets is a model for its uptake into synaptosomes and also in view of the possibility that in vivo intracellular manipulation of K^+ content could restore 5HT uptake rates to normal in D.S. platelets.

EFFECTS OF CHRONIC AMPHETAMINE ON DOPAMINE AND SEROTONIN RECEPTORS

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Amphetamine psychosis resembles acute paranoid schizophrenia and chronic amphetamine may be the best animal model for schizophrenia. Currently, the evidence suggests that dopamine receptor sensitivity may be increased in schizophrenia, thus providing a rationale for the use of dopamine receptor blockers in this mental disorder. Chronic amphetamine appears to produce an increase in behavioral response (i.e. stereotypy) following administration of dopaminergic agonists but the evidence for changes in dopamine receptors after chronic amphetamine has been contradictory. We have now re-investigated the effects of chronic amphetamine on dopamine and serotonin receptors.

Individually-housed rats were injected daily for 21 days with either 5 mg/kg or 10 mg/kg D-amphetamine sulphate. Controls received saline only. On day 23, the animals were killed. The corpus striatum and the frontal cortex were dissected out, and prepared for dopamine and serotonin receptor binding assays as described by Creese and Snyder (Eur. J. Pharmacol., 50, 459, 1978). To specifically label dopamine receptors in frontal cortex, we used a) the specific dopamine antagonist 3H-domperidone, defining specific binding as that displayed by 10 μM haloperidol. To confirm, we used 3H-spiroperidol and displaced it with unlabelled domperidone (Janssen). Serotonin (5-HT) receptor binding was determined using a) 3H-5HT (displaced by 5-HT) and b) 3H-LSD (in the presence of 30 nM spiroperidol to prevent labelling of dopamine sites) displaced with 5-HT. The results are summarized below.

			Specific binding†	
Region	Ligand (Conc.)	Control	5mg/kg	10mg/kg
Cortex	3H-5-HT (1.5nM)	37 ± 2	28 ± 1*	21 ± 2*
	3H-LSD (10nM) (+30nM spiroperidol)	108 ± 7	75 ± 10*	74 ± 20*
	3H-domperidone (0.6nM)	181 ± 11	231 ± 16*	249 ± 18*
	3H-spiroperidol (0.2nM) (displaced with 10 μM domperidone)	73 ± 5	120 ± 6*	100 ± 12*

† fmol/mg protein, mean ± S.E.M. of 4 determinations
* $p < 0.05$ compared to control.

We do not yet know if the increased binding of dopaminergic ligands and the decreased binding of serotonergic ligands represent changes in Bmax or K_D. Recently, it has become apparent that the effects of chronic amphetamine may involve the serotonergic as well as the dopaminergic system (Segal et al., Science, 207, 904, 1980). Our findings suggest that both systems are affected but in opposite directions.

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SPECIFIC BINDING OF ^{3}H-2-CHLOROADENOSINE TO RAT BRAIN CORTICAL MEMBRANES: ADENOSINE RECEPTORS

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^{3}H-2-Chloroadenosine was used as a ligand to investigate the presence of adenosine receptors on rat brain cortical membranes. Our findings have led to further insights into the mechanisms of action of adenosine as a potent depressant and the mechanisms of action of caffeine and theophylline as stimulants in CNS.

Studies on the depressant actions of adenosine and its analogs on the CNS neurons and the biochemical investigations on stimulation of cyclic AMP formation by adenosine analogs in brain slices suggest the existence of an adenosine receptor in the CNS. Therefore, evidence that shows the binding of adenosine or its analogs to the membrane will lend support to the concept of adenosine receptors.

Cerebral cortical membranes were obtained from groups of 8 male Wistar rats. The cortical homogenates were centrifuged at 4000g for 10 minutes. The pellet was resuspended and centrifuged again at 20,000g for 20 minutes. The resultant pellet was then suspended in 2.5 ml of Tris-HCl buffer (pH 7.5, containing 25mM $MgCl_2$, 25mM $CaCl_2$ and 75mM Tris). 50μl (0.5mg protein) of the rat cerebral cortical membrane was incubated with 33nM H^3-2-chloroadenosine for a period of 60 minutes at 0 ~ 4°C. At the end of incubation, 5ml of the 75mM Tris-HCl buffer solution (pH 7.5; containing 25mM of $MgCl_2$ and $CaCl_2$) was added to stop the reaction. The membranes were collected on a Whatman GF/C glass fibre filter with suction. For the determination of non-specific binding of ^{3}H-2-chloroadenosine, 2.5 x 10^{-4}M of 2-chloroadenosine was included in the incubation mixture. IC_{50} values for the inhibition of ^{3}H-2-chloroadenosine binding were obtained by adding the test agents into the incubation medium to a final concentration of 10^{-8} to 10^{-4}M. The specific binding of ^{3}H-2-chloroadenosine to rat brain cortical membranes appeared to be saturable. The specific binding was linear from 0.3mg to 1.2mg of membrane protein and it appeared to vary in different rat brain regions. Analysis of the binding data by the method of Scatchard gives a linear plot suggesting the presence of a single binding site with an apparent dissociation constant (K_D) of 23.5nM and a maximal binding capacity (B_{max}) of 476 femto mole/mg protein. The binding of ^{3}H-2-chloroadenosine to rat brain cortical membranes was inhibited by purines. The IC_{50} values were adenosine (0.12μM), adenine (0.45μM), inosine (0.4μM), theophylline (0.35μM) and isobutylmethylxanthine (0.13μM). It was weakly inhibited by uracil (18μM) and hypoxanthine (27μM). Guanosine and cytidine are inactive (> 100 μM). These findings strongly support the suggestion that there are adenosine receptors on brain cell membranes. The ability of methylxanthines to inhibit ^{3}H-2-chloroadenosine binding to the membrane preparation is also in agreement with the known activity of these agents as antagonists of adenosine action on nerve cells.

POLYELECTROMYOGRAPHIC STUDY OF EXTRAPYRAMIDAL MOTOR DISORDERS ASSOCIATED WITH NEUROLEPTICS

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A polyelectromyographic study of tardive dyskinesia and related extrapyramidal syndromes has been conducted on 24 patients.

Polygraphic recording of muscle action potentials provides objective measures of such parameters as anatomical distribution of abnormal muscular activity, intensity, type and duration of muscle contraction.

On the basis of EMG study of extrapyramidal motor disorders associated with neuroleptics three basic types of abnormal muscular activity can be readily distinguished: persistent tonic, rhythmic repetitive and phasic muscle contraction.

In some dyskinetic syndromes only one of these components can be found, while in others two or all three types of muscle contraction can be recorded. Different factors including drugs affect these three components of abnormal muscle activity in different ways.

A METHOD OF STATISTICAL ANALYSIS OF THE AMDP PSYCHOPATHOLOGY RATING SCALE BASED ON THE RESULTS OF A TWO YEAR STUDY OF TWO LONG-ACTING NEUROLEPTICS IN CHRONIC SCHIZOPHRENICS

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A two year double-blind study of 40 chronic ambulatory schizophrenics treated with either fluphenazine decanoate or pipothiazine palmitate was completed. During the first year, dosage was adjusted to the requirements of the clinical state. During the second year, the dosage remained fixed and the time period between injections was increased systematically. Results presented here deal with the data obtained from Psychopathology Rating Scale of the AMDP system. Some multivariate techniques of syndrome extraction are presented.

Principal components analysis is a multivariate tool which may effectively be used in reducing a set of syndromes from a multivariate data set. Eight syndromes identified and tentatively named on the basis of the first year's data have been subjected to meaningful analysis of therapeutic progress during both years of the study. Other methods of dealing with the problem of syndrome identification are contrasted to the analysis of principal components. It is argued that with the AMDP Psychopathology Rating Scale appropriate and conservative use of population tools in data reduction strategies may provide a systematic method of syndrome identification which is independent of the constraints of hypotheses tested in multivariate and univariate analysis of variance.

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