Interactions Between the Benzodiazepines, Methylxanthines and Adenosine

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SUMMARY: Experimental evidence is cited in support of the proposal that benzodiazepines exert their anxiolytic effects by inhibiting the uptake of adenosine by central neurons and glia.

INTRODUCTION

In spite of their well established clinical usefulness in the treatment of psychiatric, seizure and muscle spasm disorders, the sites and mechanisms of action of the benzodiazepines are as yet unknown. The recent discovery of specific benzodiazepine receptors on neuronal and glial cells (Braestrup and Squires, 1978; Henn and Henke, 1978) indicates that both types of cell may be involved. The evidence presented in this paper suggests that benzodiazepines may exert some of their actions by preventing adenosine uptake into neuronal and glial cells. The logical conclusion from our findings is that some benzodiazepine binding receptors may be associated with adenosine transport sites.

Although existing evidence points to an interaction between the benzodiazepine receptors and those for γ-aminobutyric acid (GABA) (Tallman et al., 1980), the localization of the benzodiazepine binding sites does not always coincide with those of the GABA receptors (Braestrup and Squires, 1978) and a search for additional mechanisms of action of the benzodiazepines appeared to be justified. In this context, the proposal (Klepner et al., 1979) that there are at least two biochemically distinct benzodiazepine receptor types in rat brain is of considerable interest. According to this scheme type I receptors, which are not coupled to GABA receptors, mediate the anxiolytic actions of the benzodiazepines. Type II receptors are coupled to GABA receptors and mediate other pharmacological effects such as sedation and ataxia.

It has been proposed that benzodiazepines may act by potentiating the effects of adenosine, a putative intercellular mediator in the brain (Phillis, 1979). Adenosine has a powerful depressant action on the spontaneous activity of neurons in many regions of the brain (Phillis et al., 1974; Phillis et al., 1979a) and its release from the cerebral cortex can be enhanced by electrical stimulation (Sulahke and Phillis, 1975). The effects of adenosine are antagonized by methylxanthines, such as caffeine and theophylline (Fig. 1). As these compounds are known to elicit symptoms of anxiety, it seems likely that endogenously released adenosine is involved in the regulation of anxiety-controlling centers in the brain.

Interest in the interactions between benzodiazepines and purines has been intensified by the recent report that various purines, including adenosine, inosine and hypoxanthine, can compete for a benzodiazepine-binding site in brain tissue (Marangos et al., 1979). These studies have been variously interpreted as suggesting that benzodiazepines bind to a purine receptor (Marangos et al., 1979) or to an adenosine transport site (Phillis et al., 1979b; Bender et al., 1980). Evidence that some of the actions of the benzodiazepines may be mediated by adenosine has been forthcoming from experiments on the formation of cyclic AMP in brain preparations. Benzodiazepines enhance the accumulation of cyclic AMP by a mechanism which is sensitive to adenosine deaminase and theophylline (Traversa and Newman, 1979). The results of three related studies on the adenosine-uptake-inhibiting properties of the benzodiazepines are described in the succeeding sections of this paper.

IONTOPHORETIC STUDIES

Diazepam, administered either intravenously (0.01-1 mg/kg) or iontophoretically, potentiates the depressant actions of adenosine on cerebral
cortical neurons and causes a variable amount of depression of neuronal firing (Phillis, 1979). When flurazepam was applied iontophoretically, it also potentiated the effects of adenosine and depressed the firing of cerebral cortical neurons. This depression developed slowly during a 20-30 second period and had a duration of several minutes once the application had been terminated (Fig. 2A). Flurazepam evoked depression of neuronal firing was antagonized by theophylline (Fig. 2B, C). Partial recovery of the flurazepam response occurred 1 hour after the second application of theophylline (Fig. 2D).

Evidence for a potentiative interaction between GABA and adenosine was sought in experiments on 17 rat cerebral cortical neurons. All of these cells were firing spontaneously and this firing was depressed by both compounds. There was however no indication of any synergistic interactions between GABA and adenosine. The results from one neuron are presented in Fig. 3. GABA, applied by 30nA pulses, depressed spontaneous firing. 5'-AMP was then applied for several minutes with a current of 2nA. This was sufficient to slowly depress the rate of spontaneous firing but even so there was no indication that the effects of GABA were enhanced. These observations suggest that in the cerebral cortex, which is known to contain very high concentrations of the benzodiazepine receptor, there are unlikely to be potentiative interactions between GABA and adenosine. This finding is of some interest in the light of a recent report that in cerebral cortex, unlike cerebellar cortex, chlor-diazepoxide and diazepam do not enhance GABA-evoked depressions (Assumpção et al., 1979).

**ADENOSINE AND ACETYLCHELONE RELEASE STUDIES**

To obtain in vivo evidence for an
action of diazepam at the adenosine transport site, the effect of clinically used doses of this benzodiazepine on adenosine release from the rat cerebral cortex were determined (Phillis et al., 1980b). Furthermore, since adenosine is known to affect acetylcholine (ACH) release from the cerebral cortex, the studies were extended to include ACH release. Diazepam (0.25 mg/kg) was observed to enhance the rate of efflux of (3H)-adenosine and its derivatives from the cerebral cortex and at the same time to depress ACH release. Since theophylline antagonized the effect on ACh release, there is a strong possibility that this was secondary to the increases in extracellular adenosine levels. Pentobarbital sodium and ethanol were without effect on adenosine release from the cortex and it is therefore unlikely that the diazepam effects were a result of alterations in the level of anesthesia.

ADENOSINE UPTAKE

The effects of series of benzodiazepines on the uptake of adenosine into rat brain cortical synaptosomes have been studied (Bender et al., 1980; Phillis et al., 1980a). The findings confirm earlier reports that benzodiazepines can inhibit adenosine uptake by brain preparations (Mah and Daly, 1976; Traversa and Newman, 1979). Clonazepam, nitrazepam, lorazepam, RO 11-6896, diazepam, flunitrazepam, medazepam and flurazepam, in decreasing order of potency, were the most active compounds having IC20 values of less than 10^{-6} M. The d-stereoisomer RO 11-6896 was approximatively 200 times more active than the l-isomer RO 11-6893, indicating a stereospecific interaction with the adenosine transport site. These results demonstrate that at concentrations which would be present in the brain during their therapeutic administration, the benzodiazepines can significantly reduce adenosine uptake and thus enhance extracellular adenosine levels. The potency of the benzodiazepines as adenosine uptake inhibitors shows, moreover, a good correlation with their clinical, pharmacological and receptor binding potencies (Braestrup and Squires, 1978; Mohler and Okada, 1978; Duka et al., 1979).

CONCLUSIONS

The findings of our investigations into the effects of benzodiazepines on adenosine’s actions on cerebral cortical neurons; its release from the extracellular space; and its uptake into rat cortical synaptosomes are all consistent with the hypothesis that the benzodiazepines elicit at least some of their effects by reducing adenosine uptake from the extracellular space. Adenosine actions seem to be important in those pathways which control the level of anxiety for caffeine, a potent adenosine antagonist, has a well known anxiety-provoking action and the benzodiazepines are widely used to control anxiety. The adenosine uptake rate may therefore correspond to the type 1 benzodiazepine receptor postulated by Klepner et al. (1979). A new finding which adds significant support to this proposal is the recent discovery that the adenosine uptake antagonist, dipryidamole, is a potent displacer of (3H) diazepam binding in rat brain (Davies et al., 1980). Studies with other adenosine uptake inhibitors should further strengthen our hypothesis.

REFERENCES


