Quebec Cooperative Study of
Friedreich's Ataxia

Regulation of Cytoplasmic Calcium: Interactions between Prostaglandins, Prostacyclin, Thromboxane A^2, Zinc, Copper and Taurine

D. F. HORROBIN, M. S. MANKU, S. CUNNANE, M. KARMAZYN, R. O. MORGAN, A. I. ALLY, R. A. KARMALI.

SUMMARY: *The regulation of cytoplasmic calcium is a key process in nerve tissue. Using a smooth muscle model we have shown that prostaglandin (PG) E2 probably regulates entry from extracellular fluid, whereas the release from intracellular stores depends on the interplay between thromboxane (TX) A2, PGE1 and prostacyclin. Hormones and other agents interact with this system in the following ways: vasopressin, angiotensin and inositol mobilize arachidonic acid from membrane phospholipids and increase synthesis of PGE2 and TXA2, cortisol blocks this action. Prolactin and zinc mobilize dihomo-γ-linolenic acid and increase synthesis of PGE1. These effects can be blocked by cortisol, lithium and taurine, three agents which on their own have no effect on basal PG production. Epileptogenic agents like penicillin and picrotoxin also stimulate PG synthesis, while diphenylhydantoin is a PG antagonist and diazepam is a TXA2 antagonist. The effects of all these agents occur at concentrations which are physiological in the case of the natural ones, and readily attained in human plasma in the case of the drugs. In view of recent evidence that calcium may be important in demyelination and considering the established role it plays in nerve conduction and synaptic transmission, we suggest that these observations may be of significance in understanding Friedreich's ataxia.*

RÉSUMÉ: *La régulation du calcium cytoplasmique est le processus clé dans le tissu nerveux. Grâce à un modèle expérimental de muscle lisse, nous avons démontré que la prostaglandine (PG) E2 contrôle probablement l'entrée du calcium à partir des liquides extracellulaires, tandis que la libération des réserves intracellulaires dépend de l'interaction entre la thromboxane (TX) A2, PGE1 et la prostacycline. Les hormones et d'autres agents agissent sur ce système de la façon suivante: la vasopressine, l'angiotensine et l'inositol mobilisent l'acide arachidonique à partir des phospholipides membranaires et augmentent la synthèse de PGE2 et TXA2: le cortisol bloque cette action. La prolactine et le zinc mobilisent l'acide dihomo-γ-linolénique et augmentent la synthèse de PGE1: ces effets peuvent être bloqués par le cortisol, le lithium et la taurine, trois agents qui n'ont pas d'action propre sur la production basale de PG. Les agents épileptogènes tels la pénicilline et la picrotoxine stimulent également la synthèse des PG, cependant que le diphenylhydantoin est un antagoniste des PG et le diazepam un antagoniste de la TXA2. Les effets de tous ces agents se produisent à des concentrations physiologiques pour les agents naturels et des concentrations facilement atteintes dans le plasma humain pour ce qui est des drogues. Etant donné l'évidence récente favorisant un rôle du calcium dans la demyelination et son rôle connu dans la conduction nerveuse et la transmission synaptique, nous suggérons que ces observations pourraient être utiles à la compréhension de l'ataxie de Friedreich.*

From the Clinical Research Institute of Montreal.

Reprint requests for the complete supplement on Friedreich's ataxia (Phase Two, Part One) to: Dr. André Barbeau, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7.

INTRODUCTION

The regulation of the cytoplasmic calcium concentration is emerging as a key factor in the control of cell function (Berridge, 1975; Rasmussen et al, 1975). Calcium may enter the cytoplasm from the extracellular fluid or from intracellular stores associated with membranes, sarcoplasmic reticulum or mitochondria. It may be removed by active uptake into intracellular stores or active extrusion across the outer cell membrane. The importance of calcium in the regulation of nerve conduction and synaptic transmission is central. Recent evidence suggests that calcium may play a role in diseases of myelination. Exposure of rat sciatic nerves in vitro to a calcium ionophore causes, within 30 minutes, changes in myelin previously thought characteristic of autoimmune demyelination (Schlaepfer, 1977).

While there is no doubt that the control of cytoplasmic calcium is important, the difficulties of investigating it are formidable. We have adopted an indirect approach which has proved highly productive. In vitro studies of isolated contractile systems have shown that the actomyosin system fails to generate force when the calcium concentration available to it is less than 10^{-7}M, and generates maximal force when the concentration is between 10^{-6} and 10^{-5}M. The central part of the curve relating force to log calcium concentration is linear. If other conditions are kept constant, therefore, the force generated by a muscle

https://doi.org/10.1017/S0317167100024835 Published online by Cambridge University Press

is proportional to the cytoplasmic calcium concentration.

We have chosen the isolated perfused mesenteric vascular bed of the rat as our model of calcium regulation. In this preparation injections of potassium and of vasopressin cause contractions of vascular muscle and rises in pressure in the perfusion cannula by promoting calcium entry from the extracellular fluid, since these agents cease to work within a few minutes of switching to a calcium-free medium (Manku, Horrobin, 1976). Responses to potassium and vasopressin can be used as indicators of the amount of calcium entering the cell. The time course of the decay of the response gives a measure of calcium removal, though it does not distinguish between intracellular sequestration and pumping out across the muscle cell membrane. Responses to angiotensin and noradrenaline, in contrast, are in large part independent of extracellular calcium entry and depend primarily on release of calcium in intracellular stores. If we perfuse the preparation with buffer containing only 0.25 mM calcium (a tenth of the usual amount) then responses to these two agents are almost entirely dependent on intracellular calcium release.

This preparation has a number of advantages over other smooth and skeletal muscle preparations. The baseline remains steady for hours and the response amplitude can be measurely precisely to the nearest mm Hg. Because the preparation is continuously perfused via its own blood vessel, agents being tested are uniformly distributed and washing out of substances presents no difficulties. Finally, the availability of two types of agents with sharply different requirements for intracellular and extracellular calcium allows the source of calcium in a given response to be readily identified.

How valid is the preparation as a model for other tissues such as nerve? The ability of potassium to promote calcium entry seems to be a feature of almost every tissue tested, and so we are reasonably confident that agents which modify the response to potassium in this tissue are likely to do so in others. In contrast, agents which act by releasing intracellular calcium do so by combining first with specific receptors, probably mainly on the cell surface. Each cell type seems to have a battery of such receptors and their presence or absence determines whether a cell will respond to a particular agonist. However, once this receptor/agonist combination has occurred, it seems possible that the following sequence of events leading to intracellular calcium release may have features which are common to all agonists acting on all tissues. We think agents which can modify responses to both noradrenaline and to angiotensin, which act by different surface receptors but which both release intracellular calcium, are likely to act similarly in other situations. Such extrapolation must always be cautious and is sometimes unjustified.

Roles of Prostaglandins and Related Substances

Drugs which inhibit prostaglandin biosynthesis, such as indomethacin and aspirin, block responses to all pressor agents in the preparation (Horrobin et al., 1974: Manku, Horrobin, 1976). With the indomethacin still present, prostaglandin E2 can restore responses to all agents, including potassium, noradrenaline and angiotensin. We concluded that some product of PG synthesis was necessary for calcium movement inwards across the extracellular membrane and also for intracellular calcium release, and that PGE2 could replace this endogenous PG. Later we found that imidazole, a selective inhibitor of the formation

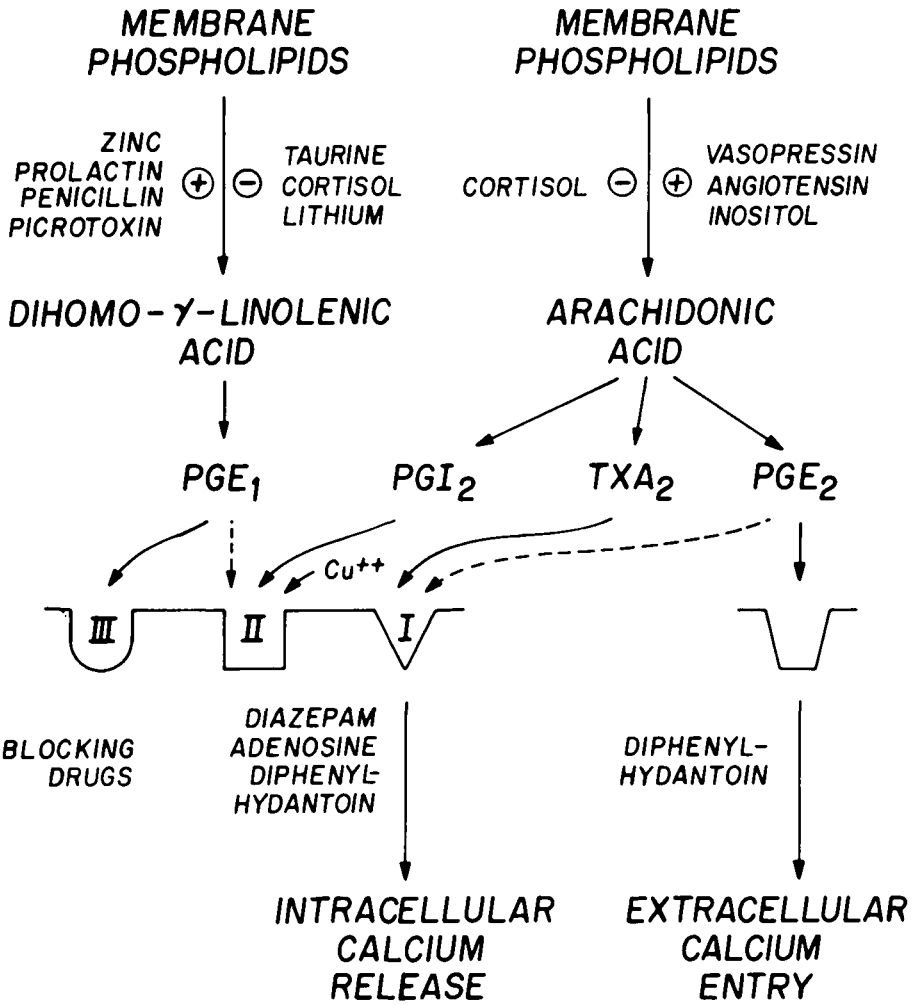

Figure 1—An outline of the pathways of prostaglandin biosynthesis and action, showing the points at which various agents act.

https://doi.org/10.1017/S0317167100024835 Published online by Cambridge University Press

of thromboxane A2 from PG endoperoxides, blocks only responses to noradrenaline and angiotensin without inhibiting those to potassium (Ally et al., 1977a). On the basis of this evidence we came to the conclusion that PGE2 was probably the natural prostaglandin regulating calcium entry across the extracellular membrane, whereas thromboxane A2 was essential for intracellular calcium release. We have also concluded that TXA2 plays an important role in promoting calcium removal from the cytoplasm at the end of a contraction, although whether this is by promoting sequestration or pumping out across the cell membrane we do not know.

Of the other natural prostaglandins we believe that the important ones are probably prostacyclin (PGI2) and prostaglandin E1 (fig. 1). PGI2 inhibits responses to noradrenaline and angiotensin, but not those to potassium (Ally et al., 1977b). We have now come to the conclusion that PGI2 can occupy a separate binding site, which non-competitively blocks the function of the TXA2 receptors.

The effects of PGE1 are complex. At very low concentrations it potentiates the responses to noradrenaline and angiotensin, but as the concentration is raised this potentiation disappears and inhibition occurs (Manku, Mtabaji, Horrobin, 1977). There are no effects of PGE1 on potassium responses at any reasonable concentration. Pharmacological analysis of the interactions between PGI2, TXA2, PGE2 and PGE1 has led us to propose that PGE1 has two binding sites. The first, which is activated by low PGE1 concentrations, non-competitively activates the TXA2 receptors. The second is identical with the PGI2 binding site. We conclude (fig. 2) that three different receptors regulate intracellular calcium release in response to activating agents.

Effects of Hormones

We have investigated the effects of angiotensin, vasopressin and prolactin in physiological ranges of concentrations on the preparation. Angiotensin and vasopressin, at concentrations which have no direct pressor effects, stimulate responses to both noradrenaline and potassium (Kondo et al. 1977: Karmazyn et al., 1978). The effects of these hormones can be imitated by arachidonic acid and, by analogy with observations in other tissues, we suggest that they activate an enzyme which splits arachidonic acid from membrane phospholipids. This leads to stimulation of both TXA2 and PGE2 synthesis and enhancement of both intracellular calcium release and extracellular calcium entry.

Prolactin enhanced responses to noradrenaline at low concentrations, inhibited them at high ones (Manku, Nassar, Horrobin, 1973) and had no effect on potassium responses. These actions were similar to those of PGE1 and could be imitated by dihomo-γ-linolenic acid. We suggest that prolactin specifically activates the release of dihomo-γ-linolenic acid from phospholipids.

Cortisol in physiological concentrations could block the responses to all three hormones (Horrobin, Mtabaji, Manku, 1976), but not those to arachidonic or dihomo-γ-linolenic acids. We believe that it blocks the release of the PG precursor fatty acids from membrane phospholipids. Lithium at concentrations used in human plasma in the treatment of manic-depressive illness eliminated the effects of prolactin (Horrobin, Karmali et al, 1976). but only partially reduced those of angiotensin and vasopressin (Karmazyn et al., 1978). We suggest that lithium has a selective effect in blocking dihomo-γ-linolenic acid mobilization.

Effects of Zinc, Copper and Taurine

There is evidence that prolactin may block zinc uptake in a wide range of tissues (Schoonees et al, 1970). Infusion of zinc had effects similar to those of prolactin, dihomo-γ-linolenic acid and PGE1: no effects on potassium, a potentiation of noradrenaline responses at low concentrations and an inhibition of noradrenaline reponses at high concentration. Prolactin causes release of ^{65}Zn from tissues prelabelled with the isotope. Combination of prolactin with its receptors

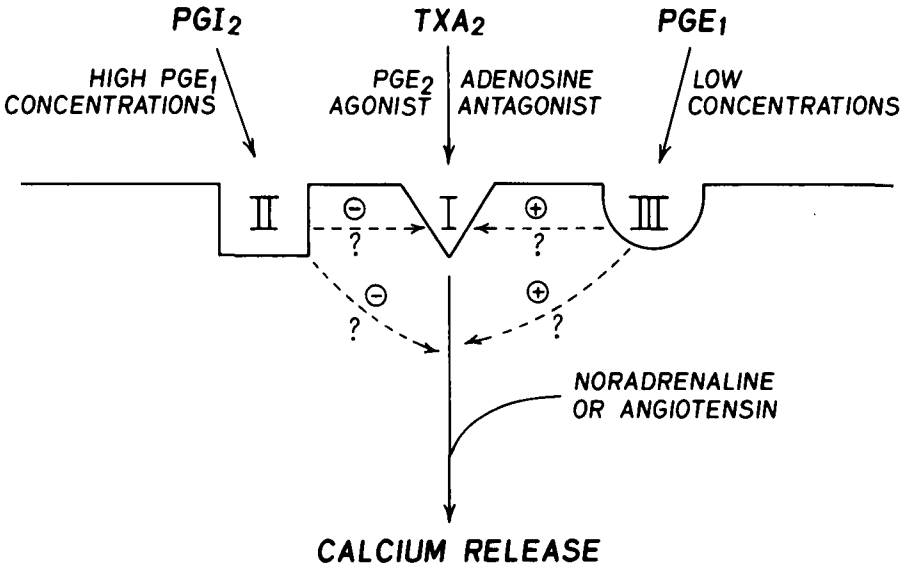

Figure 2—The regulation of intracellular calcium release by prostaglandins. At least three receptor sites seem to be involved. Activation of type I is required if calcium is to be released in response to noradrenaline or angiotensin. The type I site is normally activated by thromboxane A_2, but PGE_2 is also an agonist: adenosine is a natural antagonist. Type II receptors non-competitively inhibit the function of type I receptors. Type II receptors are activated by PGI_2 (prostacyclin) or by high PGE_1 concentrations. Type III receptors which activate type I receptors can be occupied by low PGE1 concentrations.

https://doi.org/10.1017/S0317167100024835 Published online by Cambridge University Press

may lead to release of zinc which is responsible for at least some of the effects of prolactin, including the stimulation of PGE1 synthesis.

An interaction between taurine and zinc has been proposed in the nervous system, with particular reference to the initiation of epilepsy (Barbeau and Donaldson, 1974). We could find no effect of taurine alone on responses to either noradrenaline or potassium, but it was able to prevent or to reverse the effect of zinc on noradrenaline responses. We tentatively conclude that taurine may be able to block the mobilization of dihomo-γ-linolenic acid brought about by zinc.

Interactions between zinc and copper in various biological situations have been reported. We found that copper in physiological concentrations had actions which were indistinguishable from those of PGI2 or very high concentrations of PGE1. In the presence of copper the PGI2 dose response curve moved in parallel to the left, indicating that the two were interacting at a common point. Activation of the PGI2 receptor may lead to the release of copper.

Other Agents

We have also investigated diphenylhydantoin, diazepam, chlordiazepoxide, picrotoxin, penicillin or inositol. All these agents have actions at concentrations which are physiological in the case of inositol (Karmazyn et al., 1977) or clinically readily attainable in human plasma in the case of the drugs. Diphenylhydantoin behaves as a mixed TXA2-PGE2 competitive antagonist. The benzodiazepines behave as specific TXA2 antagonists. Penicillin and picrotoxin stimulate the production of PGE1-like material. Inositol is a major component of brain phospholipids, an essential growth factor for mammalian cells and a substance which may be released as a consequence of activation of acetyl choline receptors. It seems to mobilize arachidonic acid and potentiate responses to both noradrenaline and potassium.

Conclusions

The study of the regulation of intracellular calcium in a vascular smooth muscle preparation has provided new insights into the possible modes of action of many drugs and natural substances. The effects are consistently observed at the concentrations of drugs or natural substances found in human plasma. An outline summary is shown in fig. 1.

REFERENCES

ALLY, A. I., BARRETTE, W. E., CUNNANE, S., HORROBIN, D. F., KARMALI, R. A., KARMAZYN, M., MANKU, M. S., MORGAN, R. O., NICOLAOU, K. C. (1978). Prostacyclin inhibits vascular smooth muscle contractions requiring calcium release from intracellular stores but not those requiring extracellular calcium entry. J. Physiol. in press. (a).

ALLY, A. I., MANKU, M. S., HORROBIN, D. F., MORGAN, R. O., KARMAZYN, M., and KARMALI, R. A. (1977). Dipyridamole: a possible potent inhibitor of thromboxane A2 synthetase in vascular smooth muscle. Prostaglandins 14, 607-610. (b).

BARBEAU, A., and DONALDSON, J. (1974). Zinc, taurine and epilepsy. Arch. Neurol. 30, 52-58.

BERRIDGE, M. J. (1975). The interaction of cyclic nucleotides and calcium in the control of cellular activity. Adv. Cyclic Nucleotide Res. 6, 1-65.

HORROBIN, D. F., KARMALI, R. A., MTABAJI, J. P., MANKU, M. S., and NASSAR, B. A. (1976). Prolactin and mental illness. Postgrad. Med. J. 52, suppl. 3, 79-85.

HORROBIN, D. F., MANKU, M. S., KARMALI, R. A., NASSAR, B. A., and DAVIES, P. (1974). Aspirin, indomethacin, catecholamine and prostaglandin intereactions on rat arterioles and rabbit hearts. Nature 250, 425-427.

HORROBIN, D. F., MTABAJI, J. P., and MANKU, M. S. (1976). Physiological cortisol levels block the inhibition of vascular reactivity produced by prolactin. Endocrinology 99, 406-410.

KARMAZYN, M., HORROBIN, D. F., MANKU, M. S., KARMALI, R. A., MORGAN, R. O., and ALLY, A. I. (1978). Myo-Inositol in physiological concentrations stimulates production of prostaglandin-like material. Prostaglandins, in press.

KARMAZYN, M., MANKU, M. S., and HORROBIN, D. F. (1978). Changes of vascular reactivity induced by low vasopressin concentrations. Interactions with cortisol and lithium and possible involvement of prostaglandins. Endocrinology, in press.

KONDO, K., MANKU, M. S., HORROBIN, D. F., BOUCHER, R., and GENEST, J. (1977). Potentiation of pressor effects of noradrenaline and potassium ions in rat mesenteric arteries by physiological concentrations of angiotensin II: effects of prostaglandin E2 and cortisol. Clin. Sci. Mol. Med., 53, in press.

MANKU, M. S. and HORROBIN, D. F. (1976). Indomethacin inhibits responses to all vasoconstrictors in the rat mesenteric vascular bed: restoration of responses by PGE2. Prostaglandins 12, 369-388.

MANKU, M. S., MTABAJI, J. P. and HORROBIN, D. F. (1977). Effects of prostaglandins on baseline pressure and responses to noradrenaline in a perfused rat mesenteric artery preparation: PGE1 as an antagonist of PGE2. Prostaglandins 13, 701-710.

MANKU, M. S., NASSAR, B. A. and HORROBIN, D. F. (1973). Effects of prolactin on the response of rat aortic and arteriolar smooth muscle preparations to noradrenaline and to angiotensin. Lancet 2, 991-994.

RASMUSSEN, H., JENSEN, P., LAKE, W., FRIEDMANN, N., GOODMAN, D. B. P. (1975). Cyclic nucleotides and cellular calcium metabolism. Adv. Cyclic Nucleotide Res., 5, 375-394.

SCHLAEPFER, W. W. (1977). Vesicular disruption of myelin simulated by exposure of nerve to calcium renophere. Nature, 265, 734-736.

SCHOONEES, R., DE KLERK, J. N., and MURPHY, G. P. (1970). The effect of prolactin on organ weights and zinc-65 uptake in male baboons. J. Surg. Oncol., 2, 103-106.