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REGULATION OF NORADRENALINE OVERFLOW IN RAT CEREBRAL CORTEX BY PROSTAGLANDIN E_2

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1 The effects of prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$ and PGI_2 and of inhibitors of prostaglandin synthesis and action, on the K^+-evoked [^{3}H]-noradrenaline ([^{3}H]-NA) overflow from rat cerebral cortex slices have been investigated.

2 PGE_2 reduced, while indomethacin (a prostaglandin synthesis inhibitor) or SC 19220 (a prostaglandin receptor antagonist) increased, the evoked overflow compared with controls.

3 The inhibition of [^{3}H]-NA overflow by PGE_2 was dose-dependently antagonized by SC 19220.

4 The results indicate that PGE_2 modulates NA release in rat cerebral cortex *in vitro*.

Introduction

Until recently, it was thought that the amount of noradrenaline (NA) released from sympathetic nerves was controlled only by the frequency of nerve impulses arriving at the nerve ending. However, it is now apparent that the quantitative release per impulse is subject to neuronal and humoral regulation via presynaptically located receptors (see Starke, 1977; Westfall, 1977). Modulators include the transmitter itself acting via presynaptic α and β-adrenoceptors, angiotensin, acetylcholine, adenosine triphosphate (ATP) and E type prostaglandins. Of these, autoregulation via presynaptic α-adrenoceptors appears to be a general phenomenon but endowment of presynaptic terminals with receptors for other modulators may be limited.

In some organs of certain species, NA release in response to nerve stimulation, or depolarization by K^+, is inhibited by prostaglandins (see Hedqvist, 1977); also, prostaglandins are released from many tissues during sympathetic nerve stimulation or infusion of adrenoceptor agonists (Ramwell & Shaw, 1970; Piper & Vane, 1971). It has thus been postulated that E type prostaglandins may regulate NA release and hence sympathetic neurotransmission (Hedqvist, 1969). The body of evidence in support of this theory has come from work on peripheral organs, but since prostaglandin release at various levels of the central nervous system (CNS) is also increased by nerve stimulation (Ramwell & Shaw, 1966; Gabrielyan & Aivazyan, 1978) and NA (Ramwell & Shaw, 1967; Hillier, Roberts & Woollard, 1976; Gabrielyan & Aivazyan, 1978) a similar central regulatory mechanism may exist.

Reports of prostaglandin E (PGE) modulation of NA release from CNS preparations are contradictory; thus Bergström, Farnebo & Fuxe (1973) showed a reduction in NA release from rat brain slices by PGE_2, similarly PGE_2 decreases NA overflow from rat hypothalamic synaptosomes (Wendel & Strandhoy, 1978); however, no PGE_2-induced reduction in NA overflow from similar preparations was observed by other workers (Roberts & Hillier, 1976; Gilbert, Davison & Wyllie, 1978). The reasons for these discrepancies are unclear, but may be due to differences in preparations and methods of evoking NA release.

In order to clarify the actions of prostaglandins on central NA release, we have studied the effects of several prostaglandins and prostaglandin antagonists on [^{3}H]-NA release from rat brain cerebral cortex slices. A preliminary account of this work was presented to the British Pharmacological Society (Hillier, Roberts & Templeton, 1980).

Methods

Methods were essentially those of Baldessarini & Kopin (1967) with minor modifications. Female Wistar rats (250 to 300 g) were stunned and decapitated, the brain removed and slabs of cerebral cortex cut. These were then chopped in two directions at 0.2 mm intervals on a McIlwain tissue chopper. The slices were gently dispersed in Krebs-Ringer Bicarbonate (KRB) of the following composition (mM): NaCl 112.54, KCl 4.75, KH_2PO_4 1.19, $MgSO_4$ $7H_2O$ 1.19,

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$NaHCO_3$ 25.0, $CaCl_2$ 2.57 and glucose 11.54, gassed with 95% O_2 and 5% CO_2, and centrifuged (200 *g*, 30 s) to remove substances leaking from broken cells. The slices were resuspended in 6 ml fresh KRB at 37°C containing nialamide (0.01 mM) and ascorbic acid (0.113 mM) and incubated for 2 min. (−)-[7-^{3}H]-NA 0.28 μCi/ml (sp. act 7.5 Ci/mmol; final concentration 1.65×10^{-8} M) was added and the incubation continued with shaking. After 15 min the slices were transferred under vacuum to glass microfibre filters (Whatman GF/C) (approx. 2 mg protein/filter) and placed in a superfusion apparatus through which fresh oxygenated KRB was drawn at 0.5 ml/min by a peristaltic pump. Fractions of KRB were collected during 3 min intervals into scintillation vials and the radioactivity estimated by liquid scintillation counting. At the end of the experiment, the slices were solubilized in 0.65 ml Soluene (Packard Co.) and radioactivity estimated by liquid scintillation counting with correction for quenching. Overflow of labelled material during each collection period was expressed as a percentage of the total radioactivity in the tissue (see Farah, Adler-Graschinsky & Langer, 1977).

In some experiments [^{3}H]-NA overflow was increased by depolarization of the slices with 30 mM K^+ KRB (Na^+ concentration was altered to compensate) during mins 39 to 45 of perfusion; Ca^{2+} was sometimes excluded from the KRB. Drugs were added to the perfusion medium from min 42 onward in experiments designed to assess their effect on spontaneous [^{3}H]-NA overflow and from min 30 in experiments where effects on K^+-evoked overflow were studied. The unstable prostacyclin (PGI_2) was added at min 30 and replaced by a fresh solution of PGI_2 at min 39 in similar experiments.

Overflow evoked by 30 mM K^+ was calculated as the overflow in the presence of high K^+ minus the spontaneous overflow during this period (extrapolated from 3 preceding control periods). Evoked release in the presence of drugs was expressed as a percentage of evoked release under control conditions. Two of the preparations in every experiment served as controls.

Drugs were added in aqueous solution with the exception of PGE_2, $PGF_{2\alpha}$, SC 19220 (1-acetyl-2-(8-chloro-10, 11-dihydrodibenz [b, f] [1, 4]oxazepine-10-carbonyl)hydrazine), U 46619 (15S)hydroxy-11α, 9α(epoxymethano)prostan-5Z, 13E dieonic acid) and indomethacin which were added in absolute ethanol, final concentrations of ethanol never exceeding 1%; prostacyclin was dissolved in 0.05 M Tris buffer, pH 12. Controls received appropriate carrier solutions, addition of which did not alter the pH of the KRB or affect the evoked release of [^{3}H]-NA. Drugs were purchased commercially with the exception of oxymetazoline hydrochloride (Merck), PGE_2, $PGF_{2\alpha}$, PGI_2, U 46619 (Upjohn) and SC 19220 (Searle) which were gifts.

Results

Overflow of [^{3}H]-noradrenaline

Overflow of [^{3}H]-NA was initially rapid, corresponding to washout of the extracellular space and of loosely bound [^{3}H]-NA but gradually stabilized, reaching a slowly declining plateau after 20 to 30 min. Experiments were started only during the plateau phase. Overflow of ^{3}H was stimulated by increasing the K^+ concentration of the perfusing medium from 4.75 to 30 mM; in the absence of Ca^{2+}, this increase in overflow did not occur, although spontaneous [^{3}H]-NA overflow was unaffected by Ca^{2+} lack.

Modulation of spontaneous and K^+-evoked overflow of [^{3}H]-noradrenaline

None of the drugs tested had any effect on the spontaneous overflow of [^{3}H]-NA at concentrations up to 2.8×10^{-5} M.

α-Adrenoceptor drugs The α-adrenoceptor agonist, oxymetazoline (10^{-6} M) reduced the K^+-evoked overflow to 67.9 ± 8.1% of control levels ($n = 6$, $P < 0.01$) while the evoked overflow was increased by the α-antagonist, dihydroergotamine (10^{-6} M) to 137.6 ± 6.9% of control ($n = 6$, $P < 0.001$).

Prostaglandin E_2, U 46619, indomethacin and SC 19220 PGE_2 (2.8 and 5.6×10^{-6} M) and U 46619 (2.85×10^{-6} M) (not shown) reduced the K^+-evoked overflow to 70.6 ± 1.7%, 77.0 ± 12.4% and 75.6 ± 12.0% of control respectively; while indomethacin (5.6×10^{-5} M), a prostaglandin synthesis inhibitor, and SC 119220 (6.04×10^{-5} M), a prostaglandin receptor antagonist, increased the overflow to 139.3 ± 10.8% and 137.5 ± 9.7% of control respectively (Figure 1).

When endogenous synthesis of prostaglandins was prevented by indomethacin (5.6×10^{-5} M) (Figure 2), PGE_2 was more effective in reducing the K^+-evoked overflow (compared to controls receiving indomethacin only) than when given in the absence of the synthesis inhibitor. Maximum inhibition of overflow (to 50 to 55% of control) was obtained with 2.8×10^{-6} M PGE_2. Further increase in PGE_2 concentration did not further augment the inhibition.

The reduction of [^{3}H]-NA overflow by PGE_2, in the presence of indomethacin, could be counteracted, in a dose-dependent manner, by SC 19220 (Figure 3). At the higher SC 19220 concentrations, overflow in the presence of PGE_2 was not different from control

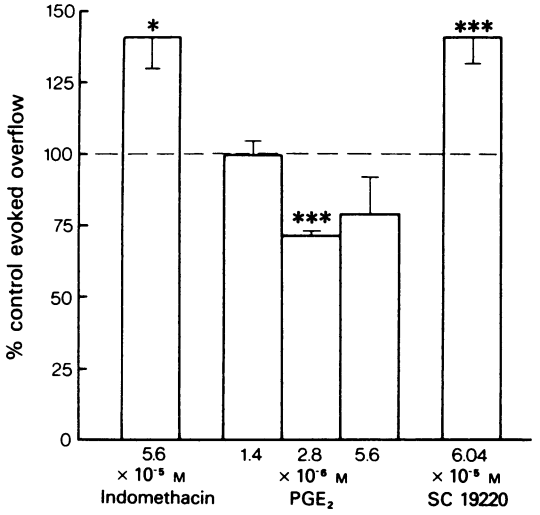

Figure 1 Effects of indomethacin, prostaglandin E_2 (PGE_2) and SC 19220 on the K^+-evoked overflow of [^{3}H]-noradrenaline from cerebral cortex slices. Results are expressed as a percentage of the evoked overflow in control samples. Each column represents the mean of not less than 4 experiments with vertical bars indicating s.e. mean. Statistics in this and subsequent figures by Student's t test. $*P < 0.05$; $***P < 0.001$.

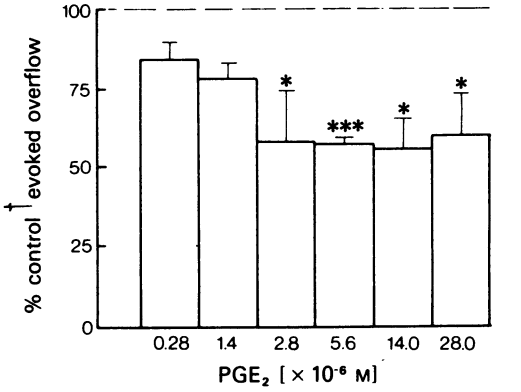

Figure 2 Effect of prostaglandin E_2 (PGE_2) on the K^+-evoked overflow of [^{3}H]-noradrenaline from cerebral cortex slices pretreated with indomethacin (5.6×10^{-5} M). Results are expressed as a percentage of evoked overflow from control samples also pretreated with indomethacin†. Each column represents the mean of not less than 5 experiments with vertical bars indicating s.e. mean. $*P < 0.05$; $***P < 0.001$.

evoked overflow. The converse was also true in that the block by SC 19220 of PGE_2-mediated inhibition of overflow could be overcome by increasing the concentration of PGE_2 present (not shown).

Prostaglandin $F_{2\alpha}$ and prostacyclin $PGF_{2\alpha}$ (doses up to 2.8×10^{-5} M) or PGI_2 (doses up to 3.03×10^{-5} M) in the absence or presence of indomethacin had no effect on K^+-evoked [^{3}H]-NA overflow.

Discussion

Our results indicate that PGE_2 can decrease K^+-evoked [^{3}H]-NA overflow from rat brain slices. Additionally, indomethacin, a prostaglandin synthesis inhibitor, and SC 19220, a prostaglandin receptor antagonist, increase the evoked overflow of [^{3}H]-NA indicating that endogenous prostaglandins act to modulate NA release. Moreover, since exogenous PGE_2 is a more effective inhibitor of [^{3}H]-NA overflow when prostaglandin synthesis is blocked by indomethacin, a background of endogenous prostaglandins may already be inhibiting overflow thus rendering the additional effect of exogenous PGE_2 less marked. It is interesting to note that the inhibition of overflow reaches a maximum at approximately 50 to

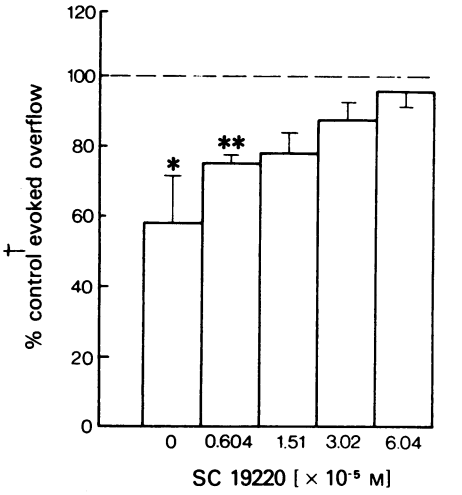

Figure 3 Reversal of the prostaglandin E_2 (PGE_2)-mediated inhibition of K^+-evoked [^{3}H]-noradrenaline overflow in indomethacin pretreated cerebral cortex slices by SC 19220. All test samples received PGE_2 (2.8×10^{-6} M) and indomethacin (5.6×10^{-5} M). Each column represents the mean of at least 5 experiments with vertical bars indicating s.e. mean. Statistical comparisons are with indomethacin-treated control samples†. $*P < 0.05$; $**P < 0.01$.

55% of control overflow, perhaps due to compensation by other control mechanisms or to saturation of prostaglandin receptor sites.

That PGE_2 may limit NA release by action at a receptor is indicated by the reversal of PGE_2-induced inhibition of overflow by SC 19220. This drug is a competitive antagonist of many smooth muscle effects of PGE_2 (Sanner, 1969; Bennett & Posner, 1971) and, to a lesser extent, $PGF_{2\alpha}$ (Bennett & Posner, 1971). The effects of SC 19220 are both organ- and species-specific (Sanner, 1974) which may explain its failure to block PGE_2 reduction of NA release from guinea-pig vas deferens (Ambache & Zar, 1970; Hedqvist & von Euler, 1972). If a PGE receptor is involved in the reduction of [^{3}H]-NA overflow, then the inhibition of evoked overflow by U 46619, a compound with potent thromboxane A_2-like activity, may be due to an interaction with the same receptor. The lack of effect of $PGF_{2\alpha}$ and PGI_2 suggests some degree of selectivity and a study of structure-activity relationships could prove useful.

In the peripheral nervous system, the PGE-mediated negative feedback of NA release appears to be less effective than the α-adrenoceptor feedback (Starke, 1977) since the enhancement of release following inhibition of prostaglandin synthesis is only 10 to 40% compared with 400% after presynaptic α-receptor blockade (see e.g. Stjarne, 1972). This difference in magnitude does not appear to hold true in the CNS where 10^{-6} M dihydroergotamine and 5.6×10^{-5} M indomethacin both produce approximately 40% increase in evoked overflow. Similarly, 10^{-6} M oxymetazoline and 2.8×10^{-6} M PGE_2 both reduce evoked overflow by approximately 40%. Also, in the peripheral system, PGI_2 is effective in reducing NA release, but is more than 100 times less potent than PGE_2 (Wennmalm, 1978). PGI_2 was ineffective in the present study, but its instability makes dose levels attained at the tissue uncertain and it was not possible to increase the PGI_2 dose to 100 times the effective PGE_2 dose for close comparison with the peripheral system.

The present results are in agreement with those of Shenoy & Ziance (1979) who observed a reduction in evoked overflow of [^{3}H]-NA from rat brain slices by PGE_2. PGE_2 also reduced [^{3}H]-NA release from hypothalamic synaptosomes (Wendel & Strandhoy, 1978); they are, however, in contrast to the negative results previously obtained (Gilbert *et al.*, 1978; Hillier, Roberts & Templeton, 1979) in rat brain cortex synaptosomes.

Evoked overflow from synaptosomes is much less than from slices stimulated with similar K^+ concentrations (Hillier and Templeton, unpublished observations); it will, therefore, be more difficult to demonstrate small changes in synaptosomes, which may explain the reports of PGE_2 ineffectiveness in the latter. This difficulty may be partially overcome by using hypothalamic synaptosomes (Wendel & Strandhoy, 1978) since the rich adrenergic innervation of this region confers a high degree of sensitivity to adrenergic control mechanisms.

Since PGE_2 does appear to reduce adrenergic overflow from brain preparations under favourable conditions, it is tempting to propose that the mechanism may operate *in vivo*; however, data are scarce and contradictory; Bergstrom *et al.* (1973) found that PGE_2 reduced NA release from rat brain while PGE_2 did not affect NA release from dog brain (Von Voigtlander, 1976) *in vivo*. Further information is thus required to confirm any physiological role for PGE_2 in cerebral cortex.

W.W.T. is an M.R.C. scholar.

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