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ACTIONS OF PROSTAGLANDINS E_1, E_2, AND $F_{2\alpha}$ ON BRAIN STEM NEURONES

BY

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The name prostaglandin was first used by Euler (1935) to describe a pharmacologically active substance present in extracts of prostate glands and semen. Prostaglandins are now known to be a family of compounds, widely distributed in mammalian tissue (see reviews by Samuelsson, 1965, and Horton, 1965). They were first isolated in a pure form by Bergström & Sjövall, 1957, 1960a and b) and their structure determined by Bergström, Ryhage, Samuelsson & Sjövall (1962).

Interest in prostaglandins in relation to the central nervous system (CNS) is of recent origin although Euler (1936 and personal communication) had hinted of an action related to sexual function. Horton (1964) was the first to produce evidence for an action of prostaglandins on the CNS and this was followed by the detection of $F_{2\alpha}$ in bovine brain by Samuelsson (1964). It seems probable that a prostaglandin was the active principle in extracts of brain that contained a pharmacologically active unsaturated hydroxy fatty acid (Ambache, Reynolds & Whiting, 1963) and also in extracts containing unsaturated fatty acids (Kirschner & Vogt, 1961 ; Toh, 1963).

The present work was initiated as a result of the observations described above to determine whether prostaglandins had any actions when applied in the neighbourhood of single neurones by micro-iontophoresis. A brief account of this work has already been published (Avanzino, Bradley & Wolstencroft, 1966).

METHODS

Experiments were performed on adult cats of either sex which were decerebrated under halothane (Fluothane, I.C.I.) anaesthesia as described by Bradley, Dhawan & Wolstencroft (1966). Electrodes were inserted through the ventral surface of the medulla and pons or the dorsal surface after removal of the cerebellum.

The methods of making and filling the four- or five-barrelled micropipettes, and recording and counting the neuronal action potentials were similar to those previously described (Bradley *et al.*, 1966).

Since only small quantities of prostaglandins were available, PGE_1 (95% or 99% pure) was used in concentrations between 0.5 and 1.0% (*p*H 6.7 to 8.0), PGE_2 (at least 90% pure) at 0.65% (*p*H 7.5) and $PGF_{2\alpha}$ at 0.08% (*p*H 7.0). A further economy in the use of the compounds was achieved

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by placing only small amounts of solution (about 0.01 ml.) in micropipettes of smaller size than usual. Since the solutions were made by addition of very dilute NaOH and HCl to the solid compounds they contained sodium and chloride ions, and consequently the resistance of the barrels was never greater than 75 $_{a}$MΩ(E_1) or 100 MΩ(F_{2a}), and was frequently less than 50 MΩ. At a *p*H>8.0 dehydration and isomerisation of the prostaglandin molecule may occur (Samuelsson, personal communication), but many results were obtained with PGE_1 at *p*H 6.7 to 7.5. *Bis*-homo-γ-linolenic acid (91% pure—main impurity linolenic acid) was used at a concentration of 5% and linolenic acid at 10%.

Prostaglandins were expelled as anions and a similar current was always passed through a saline-containing barrel as a control for current effects. In a few cases it was difficult or impossible to distinguish between the excitatory effects of passing current through the different barrels and the results obtained from these neurones were ignored.

We are grateful to Dr D. A. van Dorp for supplies of PGE_1, PGE_2 and *bis*-homo-γ-linolenic acid; and to Dr Samuelsson for pure crystalline PGF_{2a}. Linolenic acid (98.9% pure) was obtained from Koch-Light Laboratories Ltd. Histological examination of electrode tracks was made using sections stained with thionin after fixation with 10% formol-saline and embedding in paraffin wax.

RESULTS

The region of the brain stem explored with micropipettes was the medulla and caudal pons 0 to 7 mm rostral to the obex and within 3 mm from the midline. Histological examination combined with surface measurements showed that the microelectrode usually traversed the medial reticular formation. Thus many recordings must have been made from reticular neurones.

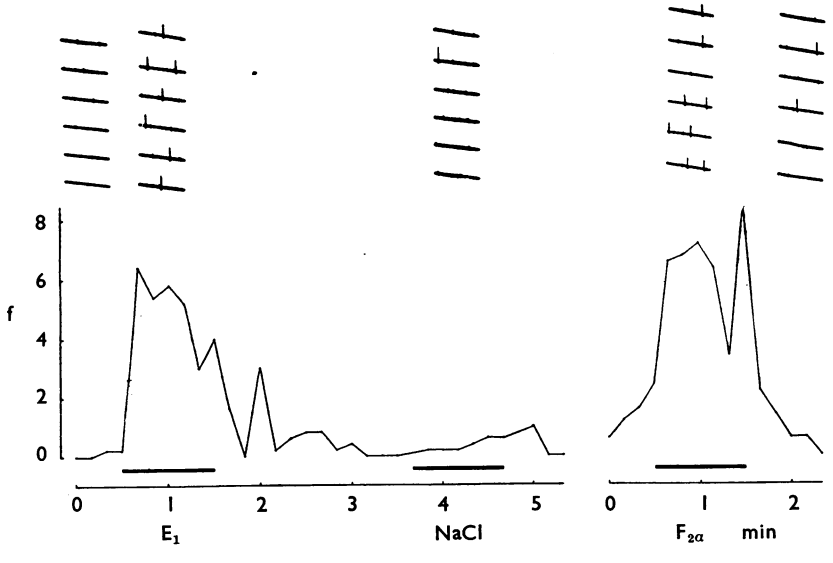

Fig. 1. The effect of PGE_1 and PGF_{2a} on the impulse frequency of a neurone in the nucleus reticularis gigantocellularis. E_1 and F_{2a} were applied with 100 nA for 30 sec. 100 nA through the barrel containing NaCl, which acted as a current control, had little effect. Above the graph are shown corresponding records of the neurone potentials, photographed from oscilloscope sweeps of 200 msec duration, triggered every 1 sec. f indicates impulses per sec.

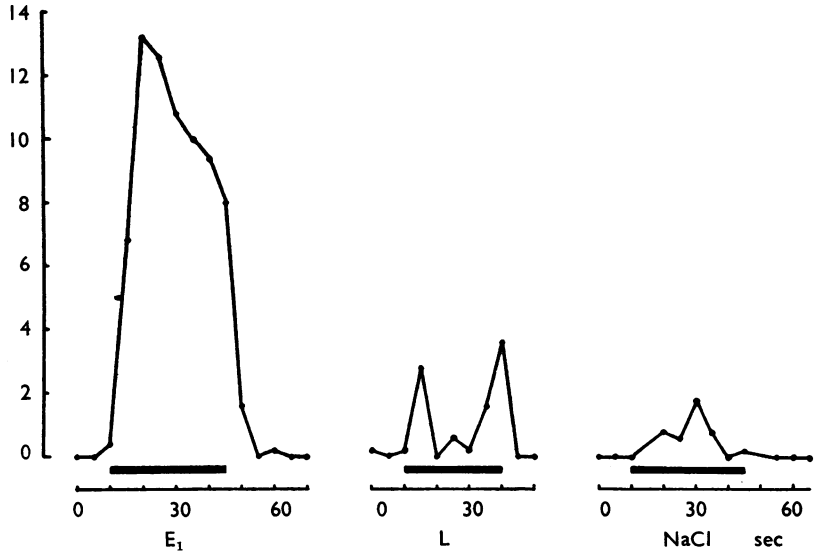

Fig. 2. Effects of PGE_1, linolenic acid (L) and current control (NaCl) all applied with 100 nA, on the impulse frequency of a medullary neurone.

Prostaglandin E_1 was tested on 341 spontaneously firing neurones, of which it excited 89 (26%) and inhibited 9 (2.5%) but did not affect the remainder. Most of these results were obtained with a sample of 95% purity but 76 of the neurones were tested with 99% pure E_1 and of these 18.5% were excited and 4% inhibited. This suggests that some of the excitatory actions of 95% pure E_1 might have been due to impurities.

Excitation, as shown in Figs. 1 and 2, was usually maximal by 5 to 10 sec and then declined, unlike the response to acetylcholine where a plateau of excitation was maintained (Bradley *et al.*, 1966). Recovery usually occurred within 10 sec. Although Figs. 1 and 2 show the action on a slow-firing neurone it should be emphasized that it was similarly effective on neurones with higher discharge frequencies.

Inhibition was slower in onset, being maximal after 20 sec in the neurone shown in Fig. 3, but recovery was rapid (within 5 to 10 sec). E_1 also inhibited neurones firing at lower rates than that illustrated in Fig. 3.

A characteristic feature of the action of PGE_1 was a reduction in, or absence of, the response to a second dose applied a short time interval after the first. Often a progressive reduction in the response was observed with successive applications 1 to 3 min apart. After a pause of 3 to 10 min there was usually partial or complete recovery. This "desensitization" was observed with most of the neurones to which repeated doses were applied, and it affected both excitatory and inhibitory responses. An example of this is shown in Fig. 3. With this neurone a further application of E_1 40 sec after the previous dose was without effect, although two applications 5 min apart (the first not shown in Fig. 3) had similar actions. During periods of desensitization the neurones still responded to other excitants such as acetylcholine or $PGF_{2\alpha}$.

Prostaglandin E_2 was tested on 69 neurones. It excited 19 (27.5%) but no inhibitory effects were found. Desensitization also occurred with this compound.

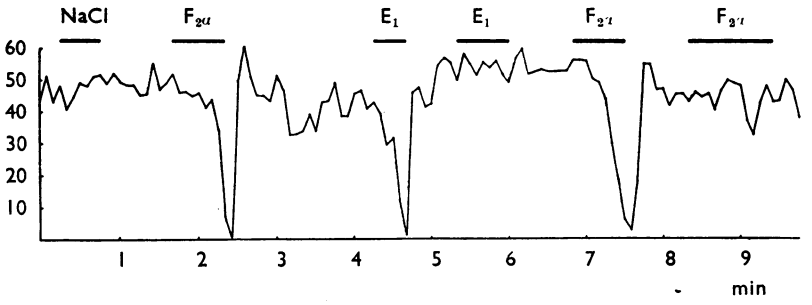

Fig. 3. Effects of E_1 (100 nA) and $F_{2\alpha}$ (100 nA) on the impulse frequency of a neurone in the nucleus reticularis gigantocellularis. Desensitization occurred with repeat doses.

Prostaglandin $F_{2\alpha}$ was tested on 155 neurones of which it excited 40 (26%) and inhibited 15 (10%). An example of its excitatory effect is shown in Fig. 1. On some occasions the action of $PGF_{2\alpha}$ was slower in onset than that shown in Fig. 1, this being possibly due to the low concentration and the preferential release of chloride ions from the solution in the micropipette.

Desensitization to both excitatory and inhibitory effects was a common finding and an example of the latter is shown in Fig. 3. The first application of $F_{2\alpha}$ reduced the firing rate almost to zero and after 4½ min a second application had a similar effect. After another 50 sec a third application had very little effect. It is interesting to note from Fig. 3 that there was no cross-desensitization between E_1 and $F_{2\alpha}$; this appeared to be a general finding. Similarly, no cross desensitization was observed between E_1 and E_2, or between E_2 and $F_{2\alpha}$.

Related compounds. *Bis*-homo-γ-linolenic acid, which has been shown to be a precursor of PGE_1 (Van Dorp, Beerthuis, Nugteren & Vonkeman, 1964; Bergström, Danielsson, Klenberg & Samuelsson, 1964) was tested on 15 neurones. It excited eight neurones, all of which responded to E_1, but the response to *bis*-homo-γ-linolenic acid was less than that to E_1 in some cases. Of the seven neurones which did not respond to *bis*-homo-γ-linolenic acid, four were excited by E_1.

Linolenic acid was also active on some neurones excited by E_1, although it was usually much less active as shown in Fig. 2.

Comparison of actions of PGE_1, E_2 and $F_{2\alpha}$. Table 1 shows the results of testing different prostaglandins on the same neurone. One of them was sometimes active when another had no effect but in no case were opposite effects found.

TABLE 1

THE EFFECTS OF TWO PROSTAGLANDINS TESTED ON THE SAME NEURONE

+ indicates excitation, − inhibition, 0 no effect. The figures indicate the number of neurones with the type of response shown above

E_1	+	+	0	−	0	0					
$F_{2\alpha}$	+	0	+	−	−	0					
	13	4	6	2	9	71					
E_1	+	0	−	0			E_2	+	0	0	
E_2	+	+	0	0			$F_{2\alpha}$	+	+	0	
	11	3	2	36				6	1	9	

TABLE 2

COMPARISON OF RESPONSES TO ACETYLCHOLINE WITH THOSE TO E_1 OR $F_{2\alpha}$ APPLIED TO THE SAME NEURONE

ACh	+	+	+	−	−	−	0	
E_1	+	0	−	+	0	−	0	
	2	8	1	3	3	1	13	
ACh	+	+	0	−	−	0	−	0
$F_{2\alpha}$	+	0	+	+	0	−	−	0
	5	4	2	1	1	3	1	12

Comparison with acetylcholine and noradrenaline. A comparison of the action of acetylcholine with those of PGE_1 and $PGF_{2\alpha}$ is shown in Table 2. In contrast to the results shown in Table 1 opposite effects on the same neurone were observed on several occasions.

No correlation was observed between the response to PGE_1 and that to l-noradrenaline in the few instances in which they were investigated.

DISCUSSION

The above results demonstrate that prostaglandins have a selective excitatory or inhibitory action when applied in very small amounts ($<10^{-5}$ μmole, assuming a transport number of <1) to brain stem neurones. It is not certain whether any prostaglandin is present in the cat brain stem, but $PGF_{2\alpha}$ has been extracted from cat fore-brain (Horton & Main, 1966) and from bovine brain (Samuelsson, 1964) and a prostaglandin-like material has been extracted from cat cerebral and cerebellar cortex (Coceani & Wolfe, 1965). A mixture of prostaglandins (tentatively identified as E_2 and $F_{2\alpha}$) is released from the somatosensory cortex following transcallosal stimulation and stimulation of the contralateral superficial radial nerve (Shaw & Ramwell, personal communication; see also Shaw, 1964, and Ramwell, 1965). It has also been found that the spontaneous release of prostaglandin into frog spinal cord perfusates is increased more than six-fold following bilateral stimulation of the hind limbs (Ramwell & Shaw, personal communication). These results suggest that one or more of the prostaglandins might have some function in relation to neuronal activity.

The negative results obtained by Krnjević ((1965) and see Horton (1965)) when PGE_1 was tested on a small sample of cortical neurones could possibly have been due to the presence of anaesthetic or to a selective action in the cerebral cortex.

The desensitization observed in the present experiments was more marked than that seen with any other compound tested by us on brain stem neurones. Little, if any, desensitization has been observed with acetylcholine but it has been found with noradrenaline (Bradley & Wolstencroft, unpublished experiments). The lack of any definite finding of cross-desensitization suggests that tachyphylaxis is very specific at the dose level used.

It is difficult to relate the results of the present experiments with the observations of Horton (1964) and Horton & Main (1965) that sedation and catatonia follow the injection of PGE_1 or E_2 into the lateral cerebral ventricles of unanaesthetized cats. However, PGE_1 and $PGF_{2\alpha}$ potentiate decerebrate rigidity in the cat when injected intravenously (Horton & Main, personal communication) and this might be related to an action on reticulospinal neurones or on other neurones which make synaptic connections with them.

In conclusion, the presence of prostaglandins in brain, their release on nerve stimulation and their excitatory and inhibitory actions on neurones in the brain stem suggest that these compounds might be chemical transmitters or that they might have some other function related to transmission in the brain. It would be interesting to know whether their presence can be demonstrated in nerve endings following subcellular fractionation (Whittaker, 1965). Caution is required however, in interpreting the above results since Ramwell, Shaw & Kucharski (1965) found that PGE_1 was liberated from the phrenic nerve-diaphragm preparation during nerve stimulation both before and after block with d-tubocurarine (but not from the nerve alone), which suggests that peripherally a prostaglandin may have functions related to transmission without actually being a transmitter substance. However, a comparison of the responses of neurones to acetylcholine, noradrenaline and prostaglandins suggests that in the brain stem the action of prostaglandins is not related to cholinergic or adrenergic transmission. Some further light may be thrown on the actions of these substances in the central nervous system when the metabolic pathways for their synthesis and degradation are known.

SUMMARY

1. The method of iontophoresis from four- or five-barrelled micropipettes was used to determine the action of prostaglandins on the spontaneous activity of neurones in the brain stem of decerebrate, unanaesthetized cats.

2. E_1 excited 18.5% and inhibited 4%, E_2 excited 27.5% but had no inhibitory effects and F_{2a} excited 26% and inhibited 10%.

3. Desensitization to both excitatory and inhibitory effects was a common finding, and it was specific for the compound applied.

4. *Bis*-homo-γ-linolenic and linolenic acids were active on some neurones excited by E_1 but inactive on others.

5. The response of a neurone to a prostaglandin was unrelated to its response to acetylcholine.

6. Neurones in the medial reticular formation of the medulla were among those excited or inhibited by prostaglandins.

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