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FURTHER OBSERVATIONS ON THE CENTRAL NERVOUS ACTIONS OF PROSTAGLANDINS $F_{2\alpha}$ AND E_1

With an Addendum on the Effects of Prostaglandins E_1 and $F_{2\alpha}$ on Systemic Arterial Blood Pressure in Chicks

BY

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We have previously reported that prostaglandins E_1, E_2 and E_3 produce catatonic stupor in the cat and sedation in the chick (Horton, 1964), and that prostaglandin $F_{2\alpha}$, the only prostaglandin so far isolated from the brain (Samuelsson, 1964), does not have these central actions (Horton & Main, 1965a). On the other hand, on intravenous injection in the young chick, prostaglandin $F_{2\alpha}$ causes dorsiflexion of the neck and extension of the legs, effects which could be either central or peripheral (Horton & Main, 1965a). It seemed likely that extension of the legs would be conducive to quantitative measurement and this was therefore used as the starting point for further investigation. From experiments reported in this paper, it is concluded that the prostaglandins act upon the spinal cord of cats and chicks. In view of the identification of prostaglandins in central nervous tissues of these species reported in the following paper (Horton & Main, 1967), the possibility that some of these actions may reflect a physiological role of the prostaglandins in the central nervous system must be considered.

Some of the experiments reported here have been demonstrated to the Physiological Society (Horton & Main, 1965b).

METHODS

Experiments on chicks

Chicks of either sex, aged from 2 days to 9 weeks old and weighing from 35 g to 1.3 kg, were anaesthetized with chloralose (60 to 80 mg/kg) or urethane (1.5 to 2 g/kg except in the preliminary experiments described under "Results,") injected intraperitoneally or, for spinal cord transection, with ethyl chloride or an ethyl chloride-diethyl ether mixture. The trachea was cannulated in all experiments and prostaglandins were injected *via* a cannula inserted into the right external jugular vein. Blood pressure was recorded from the left ischiadic artery *via* a polyethylene cannula connected to a Sanborn pressure transducer, using a Sanborn polygraph.

Mid-cervical cord transection was performed as follows. After cannulation of the trachea and right external jugular vein, the common carotid arteries, which lie deep and immediately in front of the vertebral column, were divided between ligatures. Two strong ligatures were passed round the vertebral column at about the level of the second and sixth cervical vertebrae respectively, and a third ligature was passed round the remaining tissues of the neck excluding the tracheal and jugular cannulae. The ligatures were tied and the cord sectioned between the two ligatures around

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the vertebral column at about the level of the fourth cervical vertebra. The head was then removed and subsequently weighed. The chick was artificially ventilated by a Palmer small animal respiration pump with a frequency of 36 or 48/min and delivering a volume of 2 ml./50 g chick.

Muscle tension was recorded isometrically from the freed tendon of a gastrocnemius muscle using a force-displacement transducer (Grass FT.03, 0–200 g springs). The limb was fixed to a cork board by pins inserted through the lower femur and tibia. Muscle contractions were elicited either by stimulating the cut peripheral stump of the ipsilateral sciatic nerve or, reflexly, by stimulating the cut central stump of the contralateral sciatic nerve maximally (5 V square wave pulses, 1–25 msec duration, 0.05–0.5/sec). Lower eyelid tension was recorded by a thread attached to the centre of the lid margin; in these experiments the head was immobilized. Body temperature was maintained by means of a heated operating table and an overhead warming lamp. In some but not all experiments, rectal temperature was recorded using a small thermometer.

The cerebral hemispheres were exposed by removing the overlying thin cartilaginous area of skull with fine scissors. The dura was then incised on both sides, avoiding the midline from which bleeding occurred readily. The spinal cord was exposed from the mid-thoracic to the lumbar region. Exposure of the lower lumbar region proved difficult owing to the anatomical arrangement of the avian pelvis.

Experiments on cats

Cats of either sex weighing 0.75 to 5.1 kg were either anaesthetized with chloralose (80 mg/kg injected intraperitoneally or intravenously), decerebrated at the inter-collicular level or spinalized at the level of the second cervical vertebra (with destruction of the brain) under ethyl chloride-ether anaesthesia. Spinal cats were artificially ventilated. Blood pressure was recorded from a carotid artery using a Sanborn pressure transducer. Intravenous injections were made into an external jugular vein.

Close-arterial injection to the gastrocnemius muscle. The artery supplying the gracilis muscle was cannulated with fine polyethylene tubing connected to a three-way tap. Retrograde injections were made into the femoral artery.

Recording of muscle tension. Muscle tension was recorded isometrically from the freed tendons of hind limb muscles (gastrocnemius, quadriceps and hamstring) using force-displacement transducers (Grass FT.03, 0–2 kg springs). Contractions of the gastrocnemius muscle were elicited by stimulating the cut peripheral stump of the ipsilateral sciatic nerve (5 V square wave pulses, 1 msec duration), or by direct stimulation of the muscle (20–50 V pulses, 1 msec duration) through silver wire electrodes looped round each end of the muscle. Crossed extensor reflexes were elicited from the gastrocnemius or quadriceps muscles by stimulating the cut central stump of the contralateral sciatic nerve either with single stimuli (5 V pulses, 0.1–1 msec duration) or with trains of stimuli at 20/sec lasting 5–10 sec. The patellar reflex was elicited by an electrically operated Palmer knee-jerk hammer.

Inhibition of the crossed extensor reflexes recorded from the quadriceps muscle was achieved by stretching the ipsilateral hamstring muscle, the tendon of which was connected to a force-displacement transducer. By moving the transducer through a standard distance a constant stretch could be repeatedly applied to the muscle.

Chronic denervation of the gastrocnemius muscle. The cat was anaesthetized with pentobarbitone (40 mg/kg injected intraperitoneally). Under aseptic conditions a sciatic nerve was located *via* a small skin incision on the posterior surface of the limb. A segment of nerve 1 in. long was removed, the wound closed and the cat allowed to recover. Responses of the denervated muscle were investigated 3 weeks later.

Spinal cord exposure. In decerebrate or spinal cats the spinal cord was exposed dorsally from L4 to S3. The dura was incised and reflected. In some experiments exposure was carried out under ether anaesthesia before spinalization to reduce reflex excitability. Cotton wool impregnated with a warm solution of the substance under investigation was applied to the exposed surface.

Hind limb de-afferentation. The dorsal roots from L4 to S3 on one side were cut. This abolished the crossed extensor reflex elicited by stimulating the central stump of the cut sciatic nerve on the side of the de-afferentation but not that elicited by stimulating the cut sciatic nerve centrally on the contralateral side.

RESULTS

The site of action of prostaglandin $F_{2\alpha}$ in chicks

Effects of prostaglandin $F_{2\alpha}$ on anaesthetized chicks

Preliminary experiments were made to determine whether $F_{2\alpha}$ would produce extension of the legs in the anaesthetized chick as we had observed previously in the unanaesthetized chick (Horton & Main, 1965a).

Prostaglandin $F_{2\alpha}$ (40 μg/kg) was injected intravenously in 6 two-day old chicks. Two chicks had been injected intraperitoneally with 0.9% sodium chloride solution, 2 with urethane (1.25 g/kg) and 2 with urethane (2.25 g/kg). In the unanaesthetized control chicks, $F_{2\alpha}$ caused extension of the legs lasting between 1 and 2 min, accompanied by dorsiflexion of the head and neck. The eyes were wide open. This was followed by a period during which the chick could stand with legs abducted. In the chicks lightly anaesthetized with urethane (1.25 g/kg), in which the righting reflex was absent and the eyes were shut but the withdrawal reflex to toe pinching was present, dorsiflexion of the neck and extension of the legs were observed following the intravenous injection of $F_{2\alpha}$; the eyes opened during the period when the legs were extended. In the chicks more deeply anaesthetized with urethane (2.25 mg/kg), the withdrawal reflex was absent and the intravenous injection of $F_{2\alpha}$ had no effect.

These results showed that it would be possible to use the anaesthetized chick to locate the site of action of $F_{2\alpha}$, provided that the anaesthesia was not too deep.

Fig. 1. Chicken (550 g) anaesthetized with urethane (1.7 g/kg). Record of gastrocnemius muscle tension recorded isometrically. Response to prostaglandin $F_{2\alpha}$ (1 μg) injected intravenously.

Effects of prostaglandin $F_{2\alpha}$ on gastrocnemius muscle tension

In chicks anaesthetized with urethane or chloralose, $F_{2\alpha}$ increased gastrocnemius muscle tension measured isometrically (Fig. 1). The increase in tension was similar in onset and duration to the extension of the legs observed in unanaesthetized chicks. The contraction began 10 to 30 sec after the injection and lasted up to 10 min. There was no obvious decrease in sensitivity to $F_{2\alpha}$ (on a weight basis) with age. The sensitivity to $F_{2\alpha}$ was similar in chicks anaesthetized with either urethane or chloralose. In 9 chicks

the lowest effective dose of $F_{2\alpha}$ ranged from 2 to 100 μg/kg. However, tachyphylaxis to successive doses of $F_{2\alpha}$ often developed, especially when the interval between doses was short. Assessment of threshold doses was therefore difficult.

The increase in gastrocnemius muscle tension in response to $F_{2\alpha}$ was accompanied often by defaecation and sometimes by opening of the eyes. The latter effect is illustrated in Fig. 2. In this experiment tension of the lower eyelid was recorded isometrically and the increase in eyelid tension in response to $F_{2\alpha}$ paralleled the increase in gastrocnemius muscle tension.

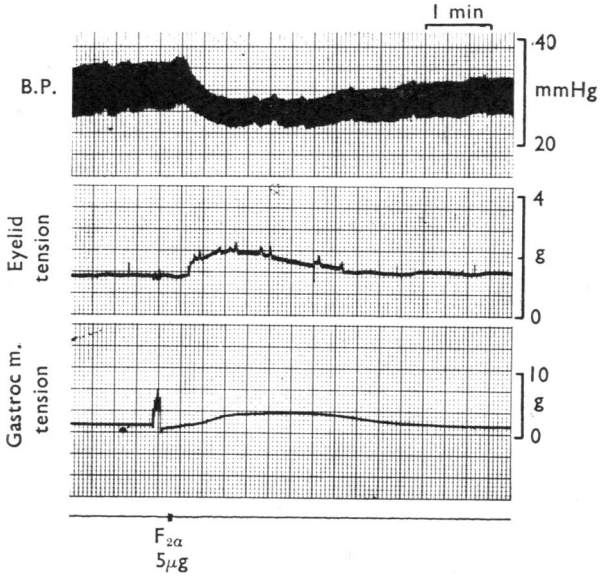

Fig. 2. Chick (85 g) anaesthetized with urethane (2.0 g/kg). Upper trace: systemic arterial blood pressure. Middle trace: lower eyelid muscle tension. Lower trace: gastrocnemius muscle tension. Responses to prostaglandin $F_{2\alpha}$ (5 μg) injected intravenously.

Effects of prostaglandin $F_{2\alpha}$ on systemic arterial blood pressure

In 4 chicks anaesthetized with urethane, in which gastrocnemius muscle tension and ischiadic arterial blood pressure were recorded simultaneously, increases in gastrocnemius muscle tension following an intravenous injection of $F_{2\alpha}$ were accompanied by changes in blood pressure which varied from one chick to another; in one chick there was a rise, in a second a fall and in the other 2 chicks a rise followed by a fall in blood pressure. In view of this it seems unlikely that either a rise or a fall of blood pressure could have been the cause of the increase in gastrocnemius muscle tension produced by $F_{2\alpha}$. Furthermore, similar rises or falls in blood pressure produced by injecting adrenaline or acetylcholine did not alter gastrocnemius muscle tension.

Effect of mid-cervical cord transection on the response to prostaglandin $F_{2\alpha}$

In 8 chicks whose spinal cord had been sectioned in the mid-cervical region (decapitated weight 31–71 g), $F_{2\alpha}$ in doses of 9 to 166 μg/kg, given intravenously, increased gastrocnemius muscle tension.

Effect of acute denervation on the response to prostaglandin $F_{2\alpha}$

In one unanaesthetized chick the left sciatic nerve was sectioned after infiltration of the overlying skin with amethocaine. Fifteen minutes after section an intravenous injection of $F_{2\alpha}$ (330 μg/kg) resulted in the usual dorsiflexion of the neck accompanied by extension of the right (innervated) limb. There was no extension of the left (denervated) limb.

In the anaesthetized or spinal chick $F_{2\alpha}$ also failed to produce a contraction if the gastrocnemius muscle was acutely denervated. In the experiment on a spinal chick illustrated in Fig. 3, both sciatic nerves were exposed and the tension of both gastrocnemius muscles

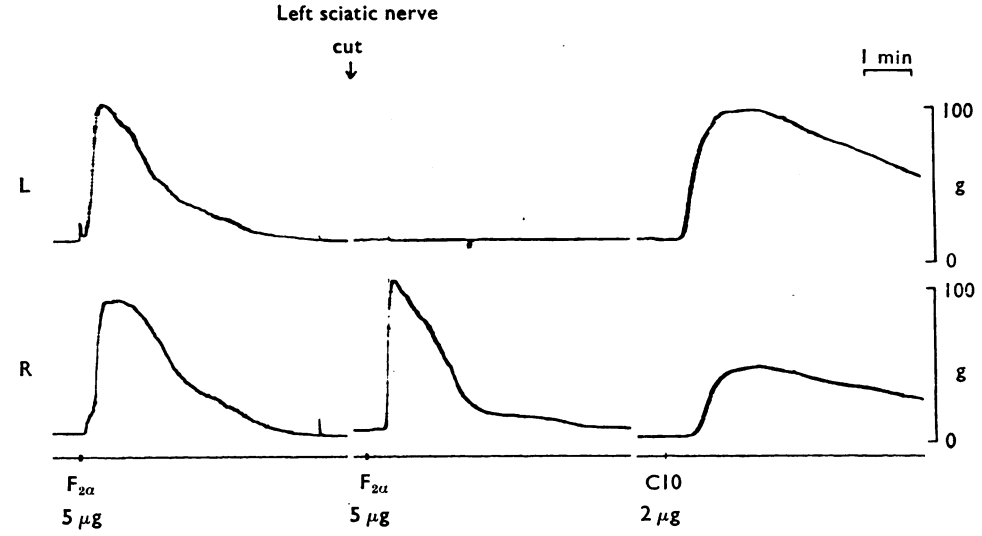

Fig. 3. Spinal chick (decapitated weight 32 g). Gastrocnemius muscle tension recorded isometrically (upper trace: left leg; lower trace: right leg). Responses to prostaglandin $F_{2\alpha}$ (5 μg) and decamethonium iodide (C10, 2 μg) injected intravenously. Between the first and second panel the left sciatic nerve was cut. There was an interval of 60 min between each injection.

was recorded. $F_{2\alpha}$ (156 μg/kg) injected intravenously increased the tension of both the left and the right gastrocnemius muscles. After the left sciatic nerve had been sectioned the same dose of $F_{2\alpha}$ increased the tension of the right gastrocnemius muscle only. To avoid tachyphylaxis the interval between doses was 1 hr. Blood pressure was not recorded in this experiment since cannulation of an ischiadic artery would have interfered with the limb blood flow and in the 2-day old chick no satisfactory alternative artery was available for cannulation. Adrenaline (16 μg/kg) was injected intravenously midway between the doses of $F_{2\alpha}$ to ensure that the blood pressure was maintained. After denervation decamethonium iodide (63 μg/kg) contracted both gastrocnemius muscles indicating that the denervated gastrocnemius muscle was still capable of contracting in response to drugs injected intravenously.

These experiments show that the response of the gastrocnemius muscle to $F_{2\alpha}$ is not due to a direct action on the muscle or an action on the neuromuscular junction.

The site of action of prostaglandins $F_{2\alpha}$ and E_1 in decerebrate and spinal cats

Experiments on decerebrate cats

Although in the unanaesthetized cat prostaglandin $F_{2\alpha}$ injected either intravenously (15 μg/kg) or into the cerebral ventricles (15–100 μg) has no obvious effect, it was observed that in the decerebrate cat $F_{2\alpha}$ injected intravenously increases gastrocnemius muscle tension as it does in the intact and spinal chick. However, in the decerebrate cat, unlike in the chick, E_1 also increases gastrocnemius muscle tension on intravenous injection. These actions are illustrated in Fig. 4. In 3 decerebrate cats the approximate threshold dose of $F_{2\alpha}$ was 5 to 7 μg/kg and in 7 decerebrate cats the approximate threshold dose of E_1 ranged from 5 to 15 μg/kg. In 2 cats E_1 in doses of 8 and 17 μg/kg failed to increase gastrocnemius muscle tension, although the fall in arterial blood pressure was the same as in the other cats. Tachyphylaxis to E_1 sometimes developed.

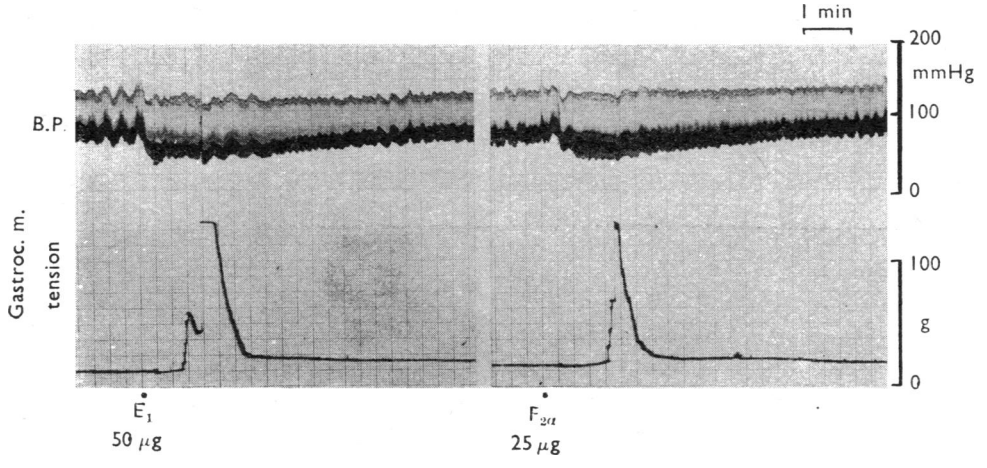

Fig. 4. Decerebrate cat (3.4 kg). Upper trace: carotid blood pressure. Lower trace: gastrocnemius muscle tension. Responses to intravenous injections of prostaglandin E_1 (50 μg) and prostaglandin $F_{2\alpha}$ (25 μg).

The interval between the injection and the onset of contraction varied from 25 to 100 sec. Sometimes the contractions of the gastrocnemius muscle were accompanied by arching of the back, extension of the forelimbs and on two occasions walking movements. Decerebrate rigidity was potentiated. E_1 and $F_{2\alpha}$ always caused a fall in systemic arterial blood pressure sometimes accompanied by an increase in ventilation rate. There was no obvious correlation between the magnitude of the depressor response and the contraction of the gastrocnemius muscle. Larger depressor responses produced by intravenous injection of acetylcholine (10 μg/kg) had no effect on gastrocnemius muscle tension.

In view of the apparent similarity in response of the gastrocnemius muscle in the decerebrate cat to E_1 and $F_{2\alpha}$, and because of the shortage of $F_{2\alpha}$, subsequent studies on the site of this action of the prostaglandins in the cat were carried out with E_1.

In the decerebrate cat acute denervation of the gastrocnemius muscle prevented the increase in tension following an intravenous injection of E_1. On close-arterial injection

into a hind limb in doses similar to those effective by the intravenous route, E_1 had no effect on gastrocnemius muscle tension. Higher intra-arterial doses sometimes increased tension but only after a longer latent period than after intravenous injection, and the response was bilateral. These results show that the increased gastrocnemius muscle tension following intravenous injection of E_1 in the decerebrate cat is not due to an action on skeletal muscle or the neuromuscular junction.

Experiments on spinal cats

In 3 spinal cats E_1 in threshold doses ranging from 5 to 40 μg/kg increased gastrocnemius muscle tension, the time course of the contraction being similar to that in the decerebrate cat. In 3 other spinal cats E_1 in doses from 16 to 58 μg/kg had no effect on gastrocnemius muscle tension.

The response of the gastrocnemius muscle to E_1 in the spinal cat was abolished by denervation of the muscle but not by dorsal root (L4 to S3) section. Close-arterial injections of E_1 into the hind limb of the spinal cat, in doses similar to those effective by the intravenous route, had no effect on gastrocnemius muscle tension, thus confirming the findings in decerebrate cats that this is not a peripheral action of prostaglandin E_1.

In one experiment a direct action of E_1 on the spinal cord was demonstrated by topical application. An increase in gastrocnemius muscle tension occurred when E_1 (500 μg/ml.) was applied to the dorsal surface of the lumbar (L6–7) cord. This effect could be elicited repeatedly. Application of saline had no effect. Concentrations of 100 μg/ml. or less of E_1 had no effect.

Action of prostaglandin E_1 on skeletal muscle

The experiments show that the increase in gastrocnemius muscle tension is not due to an action of E_1 on the muscle itself. In higher doses E_1 injected close-arterially to the muscle had an inhibitory effect on muscle twitches. In a spinal cat E_1 (29 μg/kg) injected close-arterially inhibited contractions of the gastrocnemius muscle in response to electrical stimulation of the sciatic nerve. In another experiment, on a chloralosed cat, contractures of the chronically denervated gastrocnemius muscle in response to close-arterial injections of acetylcholine (0.18 μg/kg) were also inhibited by E_1 (23 μg/kg) injected close-arterially. This effect lasted throughout the remainder of the experiment. On the other hand, contractions due to direct electrical stimulation were not significantly affected (Fig. 5).

Effects of prostaglandins $F_{2\alpha}$ and E_1 on spinal reflexes in cats and chicks

During the experiments described above a crossed extensor reflex was sometimes elicited as a test of the integrity of the spinal cord. It was observed that prostaglandins usually potentiated this reflex in both the chick and the cat. This action of prostaglandins on spinal reflexes has been investigated further and the results are described below.

Spinal chicks

In some of the early experiments on spinal chicks no crossed extensor reflex could be elicited, while in other experiments the reflex appeared only after an interval of 30–60 min. In later experiments, an intravenous injection of 0.9% sodium chloride solution or

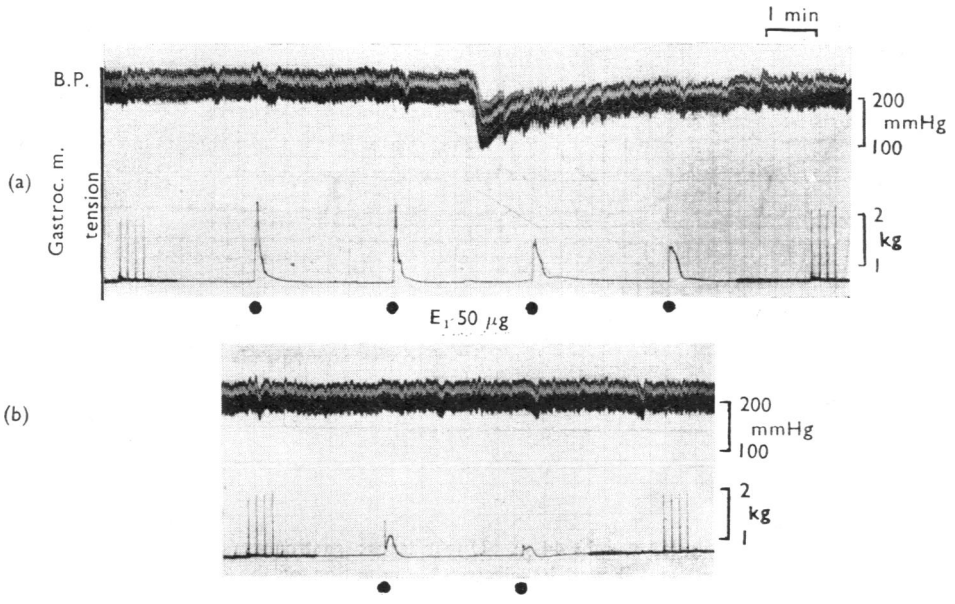

Fig. 5. Cat (2.2 kg) anaesthetized with chloralose (80 mg/kg). Upper trace: carotid arterial blood pressure. Lower traces: muscle tension recorded from a chronically denervated gastrocnemius muscle; twitches were elicited by direct electrical stimulation of the muscle; contractures were elicited by close-arterial injection of acetylcholine (0.4 μg) as indicated by the dots. The interval between (a) and (b) was 12 min. Effects of prostaglandin E_1 (50 μg) injected close-arterially.

adrenaline (5–10 μg/kg) was given immediately after section of the spinal cord, and in these experiments reflexes were present from the outset.

In the spinal chick, reflex contractions of the gastrocnemius muscle in response to stimulating the central stump of the cut contralateral sciatic nerve were potentiated by $F_{2\alpha}$ in doses too low to affect muscle tension in the absence of stimulation. The potentiation began 5–10 sec after the injection, reached a maximum within 30–60 sec and usually lasted 3–30 min according to the dose injected. The crossed extensor reflex was elicited in 16 spinal chicks weighing from 42 to 141 g. In every case $F_{2\alpha}$, in doses ranging from 2 to 150 μg/kg potentiated the response. With the higher doses of $F_{2\alpha}$, a contraction of the gastrocnemius muscle was observed in addition to the potentiation of spinal reflexes (Fig. 6), and tachyphylaxis to both responses frequently occurred.

Prostaglandin E_1 also potentiated the crossed extensor reflex in spinal chicks in threshold doses (1.5 to 5 μg/kg) similar to those of $F_{2\alpha}$. Higher doses of E_1 caused transient potentiation of reflexes and convulsive movements of the chick, but this was followed by inhibition of the reflex possibly due to the lowering of blood pressure. Unless adrenaline was injected to restore the blood pressure, death often occurred after these higher doses of E_1 in the spinal chick.

When gastrocnemius muscle twitches were elicited by stimulation of the intact ipsilateral sciatic nerve, intravenous injections of $F_{2\alpha}$ and E_1 did not affect the size of the muscle

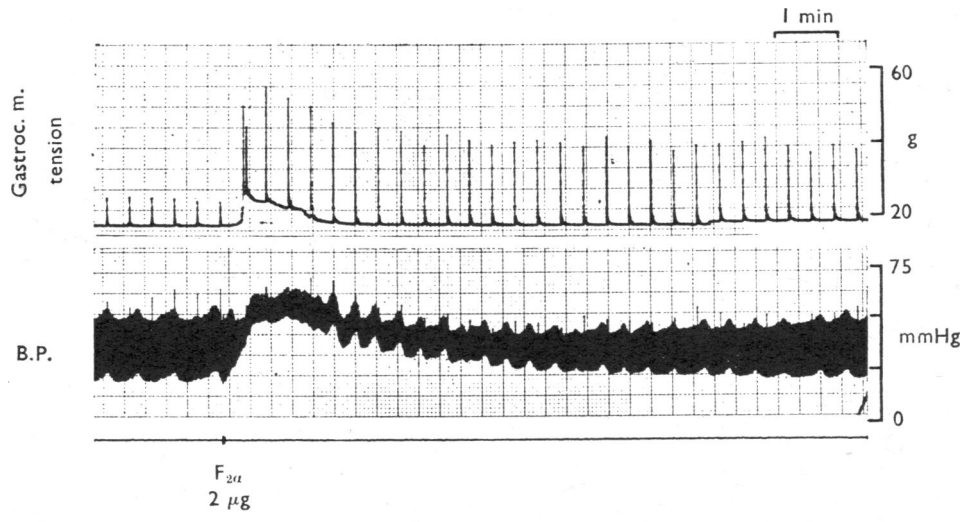

Fig. 6. Spinal chick (decapitated weight 44 g). Upper trace: gastrocnemius muscle tension, crossed extensor reflex twitches were elicited by electrical stimulation of the contralateral sciatic nerve. Lower trace: systemic arterial blood pressure. Response to prostaglandin $F_{2\alpha}$ (2 μg) injected intravenously.

twitches, either in doses which potentiated spinal reflexes or even in doses (of $F_{2\alpha}$) high enough to cause contraction of the ipsilateral gastrocnemius muscle. These results indicate that the potentiation by prostaglandin of the crossed extensor reflex is central not peripheral.

The action of $F_{2\alpha}$ and E_1 on the crossed extensor reflex of the spinal chick resembled strychnine. No valid comparison of potency of prostaglandins and strychnine could be made since the dose-response curves were not parallel. The threshold dose of strychnine was usually of the order of 10 μg/kg. The maximum potentiation which could be achieved with higher doses of strychnine was greater than that which could be produced with the prostaglandins, and the effect of strychnine lasted longer.

Anaesthetized chicks

The crossed extensor reflex could be elicited readily in chicks anaesthetized with chloralose but not in chicks lightly anaesthetized with urethane. Furthermore, small doses of urethane (0.2 g/kg) reduced or abolished the crossed extensor reflex in chloralosed chicks.

In 9 experiments on chloralosed chicks from 2 days to 9 weeks old weighing 35 g–1.25 kg, $F_{2\alpha}$, in doses ranging from 4–15 μg/kg, potentiated the crossed extensor reflex. In chloralosed chicks E_1 either had no effect or inhibited the crossed extensor reflex in doses (1–8 μg/kg) similar to those which potentiated the reflex in the spinal chick.

Neither E_1, nor $F_{2\alpha}$, when applied topically to the dorsal surface of the lumbar region of the spinal cord, had any effect on the crossed extensor reflexes. In one experiment E_1 (100 μg/ml.) had no effect whereas strychnine (100 μg/ml.) caused marked potentiation.

Topical application of E_1 (50–100 μg/ml.) to the exposed cerebral hemispheres had little or no effect on the reflex in the chloralosed chick except in one experiment in which the reflex was reduced by E_1 (100 μg/ml.). The possibility that this action may not have been a local one but was secondary to absorption into the systemic circulation cannot be entirely excluded. The effect was accompanied by a slight fall in arterial blood pressure.

Decerebrate and spinal cats

Prostaglandin E_1 potentiated the crossed extensor reflex in decerebrate and spinal cats in doses of 1–20 μg/kg and 10–32 μg/kg respectively. Figure 7 shows an experiment on a spinal cat in which E_1 caused a potentiation of the crossed extensor reflex which lasted 30 min. Sometimes potentiation of the reflex was preceded by a slight inhibition which coincided with the maximum lowering of the blood pressure (20–30 sec.). Prostaglandin E_1 had no detectable effect on the patellar reflex, in doses which potentiated the crossed extensor reflex (Fig. 7).

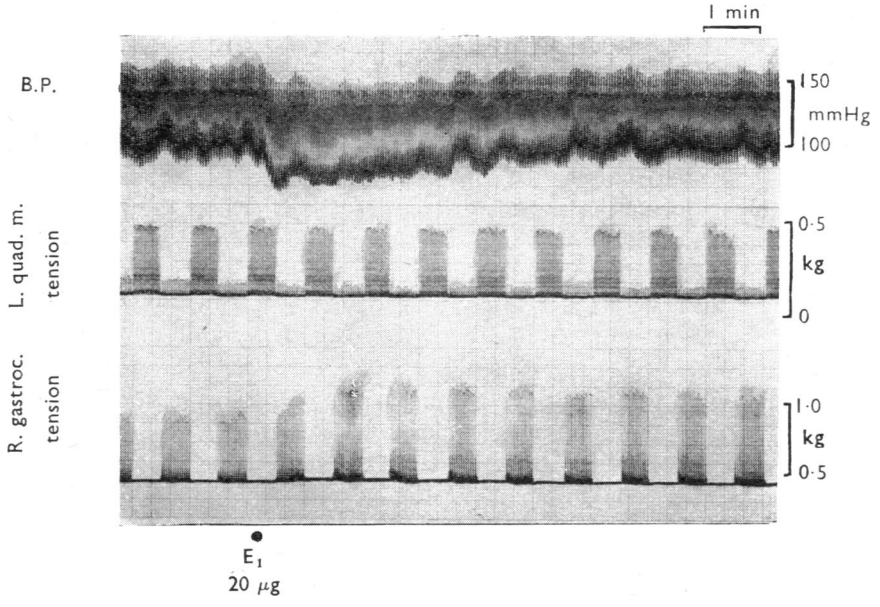

Fig. 7. Spinal cat (3.1 kg). Upper trace: carotid arterial blood pressure. Middle trace: left quadriceps muscle tension, patellar reflex twitches. Lower trace: right gastrocnemius muscle tension, twitches were elicited by stimulating the contralateral sciatic nerve. Effect of prostaglandin E_1 (20 μg), injected intravenously.

Since E_1 did not potentiate the twitches of the gastrocnemius muscle in response to ipsilateral nerve stimulation, it is concluded that the potentiation of spinal reflexes in the cat is due to an action on the spinal cord. This could be due to facilitation of the firing of motor neurones. Such an action could be either by direct facilitation, or by inhibiting pre- or post-synaptic inhibitory mechanisms. A few experiments were carried out on spinal cats in an attempt to find out which mechanism might be responsible.

In one experiment, the crossed extensor reflex, recorded from the left quadriceps muscle, was elicited by repetitive stimulation of the contralateral sciatic nerve for periods of 10 sec at 1 min intervals. A constant stretch applied to the left hamstring muscle during each reflex response resulted in marked inhibition. E_1 (30 μg/kg) potentiated the crossed extensor reflex but did not affect the degree of inhibition. The tension developed in the hamstring muscle during stretching was also increased by E_1.

In another experiment inhibition of the crossed extensor reflex by electrical stimulation of the ipsilateral peroneal nerve was unaffected by E_1.

These results suggest but do not prove that reflex potentiation of E_1 does not result from inhibition of inhibitory mechanisms.

Topical application of E_1 to the spinal cord of spinal cats, in concentrations of 20–100 μg/ml. did not affect spinal reflexes. In one experiment referred to above contraction of the gastrocnemius muscles and potentiation of crossed extensor reflexes followed the application of higher concentrations of E_1 (500 μg/ml.) to the dorsal surface of the cord in the L6–L7 region.

DISCUSSION

Evidence that prostaglandin $F_{2\alpha}$ acts on the spinal cord of the chick

Our experiments confirm that extension of the legs observed in the chick after an intravenous injection of $F_{2\alpha}$ (Horton & Main, 1965a) is due to contraction of the extensor muscles such as the gastrocnemius muscle. By recording the increase in tension of this muscle we have been able to locate the site of this action of $F_{2\alpha}$. Since the response to $F_{2\alpha}$ is abolished by acute denervation of the muscle, it must be centrally mediated. Since it is readily elicited in the decapitated chick the brain cannot be necessary for the mediation of this effect, nor can it be a reflex response mediated by pathways which depend upon the integrity of the brain stem. Reflex contraction of the gastrocnemius muscle due to $F_{2\alpha}$ acting upon spinal afferent nerves is unlikely because under light urethane anaesthesia electrical stimulation of the cut central stump of the sciatic nerve failed to elicit reflex contractions of the contralateral gastrocnemius muscle whereas $F_{2\alpha}$ injected intravenously did produce a contraction of the muscle. It is, therefore, concluded that $F_{2\alpha}$ increases gastrocnemius muscle tension by an action on neurones within the chick spinal cord. Confirmation of this conclusion by topical application of $F_{2\alpha}$ to the exposed spinal cord has not yet been obtained, possibly because the concentrations applied were too small. Potentiation by $F_{2\alpha}$ of the crossed extensor reflex in the chloralosed and spinal chick is compatible with an excitatory or facilitatory action on spinal cord neurones or of inhibition of inhibitory pathways.

Although $F_{2\alpha}$ acts upon the spinal cord of the chick, the possibility that it also acts on the brain cannot be excluded.

Evidence that prostaglandin E_1 acts on the spinal cord of the cat

Increases in gastrocnemius muscle tension in the spinal cat in response to intravenous injections of E_1 could not be imitated by injecting the prostaglandin close-arterially to the muscle. Furthermore, acute denervation of the muscle abolished the response to intravenous E_1 showing that the action of the prostaglandin cannot be upon the skeletal

muscle or the neuromuscular junction, but must be centrally mediated. Reflex contractions of the gastrocnemius muscle mediated *via* the brain could not of course account for the positive responses obtained in the spinal animal, but reflex contractions due to E_1 acting upon spinal afferent nerves are not excluded. A direct action of E_1 on the cord is more likely, because, in one experiment, contractions of the gastrocnemius muscle were, elicited by topical application of E_1 to the spinal cord. We conclude that prostaglandin E_1 in the cat, like $F_{2\alpha}$ in the chick, can increase gastrocnemius muscle tension by an action on the spinal cord.

Since the effect of E_1 was observed equally on both gastrocnemius muscles after unilateral section of the dorsal roots, it could not be due to facilitation of γ-motoneurone firing alone. We conclude that E_1 facilitates the firing of α-motoneurones either directly or indirectly. The facilitation is probably due to stimulation of excitatory pathways since inhibition of inhibitory pathways was not observed after prostaglandin administration. This problem must be investigated further using electrophysiological techniques.

Responses to E_1 observed in the decerebrate cat could be accounted for by an action on the spinal cord. The question of whether E_1 also excites hind-brain descending facilitatory pathways remains unsettled, though Avanzino, Bradley & Wolstencroft (1966) have shown that medullary reticulospinal neurones respond to prostaglandins applied iontophoretically and that the predominant response is excitation. Increases in gastrocnemius muscle tension in response to E_1 were obtained more readily in the decerebrate than the spinal animal. Whether this could be explained on the basis of additional contributions from the stimulation of descending facilitatory pathways which have their origin in the hind-brain is a matter for further experiment.

The evidence concerning the site of action of $F_{2\alpha}$ in the cat is incomplete. Certainly $F_{2\alpha}$ had a similar action to E_1 on the decerebrate cat and it may be supposed that it also exerts its effect partly, at least, on the spinal cord. Avanzino *et al.* (1966) showed that $F_{2\alpha}$ fires off reticulospinal neurones in the medulla and Duda & McPherson (personal communication) have observed that $F_{2\alpha}$ has actions on the spinal reflexes in the chloralosed cat, when injected into the abdominal aorta.

Our experiments on reflexes in both the spinal chick and the spinal cat support the conclusion that prostaglandins have facilitatory action on the firing of α-motoneurones, though the inhibitory action of E_1 on the crossed extensor reflex in the chloralosed chick is suggestive that prostaglandins may also be inhibitory or excite inhibitory pathways in certain circumstances. These observations have received some support from the recent experiments of Duda & McPherson (personal communication) on the chloralosed cat using electrophysiological recording techniques.

Possible physiological implications of these results are discussed more fully in the following paper.

SUMMARY

1. Prostaglandin $F_{2\alpha}$ on intravenous injection in chicks increases gastrocnemius muscle tension. Experiments were made to locate its site of action.

2. The effect was observed in the decapitated chick but was abolished by denervation of the muscle. It could be elicited in the urethanized chick in which reflex contractions

were blocked. It is concluded that the site of this action of $F_{2\alpha}$ in the chick is upon the spinal cord.

3. In spinal cats prostaglandin E_1 injected intravenously increased gastrocnemius muscle tension also by an action on the spinal cord. The effect was abolished by denervation of the muscle but not by dorsal root section. Close-arterial injection of E_1 to the gastrocnemius muscle did not elicit a contraction but did inhibit muscle twitches and acetylcholine-induced contractures. In one experiment prostaglandin E_1 applied topically to the spinal cord induced a contraction of the gastrocnemius muscle.

4. Decerebrate rigidity in the cat was potentiated by prostaglandins $F_{2\alpha}$ and E_1.

5. Crossed extensor reflexes were potentiated by prostaglandin $F_{2\alpha}$ in the spinal chick and by E_1 in the spinal cat. The patellar reflex in the spinal cat was little affected by prostaglandin E_1. In the chloralosed chick $F_{2\alpha}$ potentiated but E_1 inhibited the crossed extensor reflex.

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ADDENDUM

Effects of prostaglandins E_1 and $F_{2\alpha}$ on systemic arterial blood pressure in chicks

During experiments on chicks described in this paper, arterial blood pressure was usually monitored. In all preparations E_1 had a depressor effect. In spinal preparations $F_{2\alpha}$ had a pressor effect but in anaesthetized chicks the response to $F_{2\alpha}$ was more variable; sometimes pressor, sometimes depressor, and sometimes biphasic (pressor followed by depressor). The results are summarized in Table 1.

The pressor action of $F_{2\alpha}$ in spinal chicks was not abolished by phenoxybenzamine (10 mg/kg) which abolished responses to noradrenaline nor by hexamethonium (10 mg/kg) or pronethalol (10 mg/kg).

TABLE 1

ACTIONS OF PROSTAGLANDINS ON THE ARTERIAL BLOOD PRESSURE OF ANAESTHETIZED AND SPINAL CHICKS

		Number of experiments			
				Arterial blood pressure response	
Preparation	Prostaglandin	Total	Pressor	Pressor–depressor	Depressor
Spinal	$F_{2\alpha}$	14	14	0	0
	E_1	9	0	0	9
Chloralose anaesthesia	$F_{2\alpha}$	14	8	5	1
	E_1	8	0	0	8
Urethane anaesthesia	$F_{2\alpha}$	10	3	4	3
	E_1	15	0	0	15
All anaesthetized chicks	$F_{2\alpha}$	24	11	9	4
	E_1	23	0	0	23

In 3 experiments on the isolated chicken heart perfused by the method of Langendorff, $F_{2\alpha}$ had no action on the force or rate of contraction in doses higher than those which had a pressor action on intravenous injection in the spinal chick. In 2 experiments E_1 (25 and 50 μg) injected into the perfusion fluid increased the force of contraction but smaller doses had no effect.

The results suggest that the pressor action of $F_{2\alpha}$ is not mediated by the sympathetic nervous system and does not result from a direct action on the heart.

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