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The distribution of tritium-labelled prostaglandin E_1 injected in amounts sufficient to produce central nervous effects in cats and chicks

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1. After injection of tritiated prostaglandin E_1 into the lateral cerebral ventricle, the carotid and vertebral arteries and the aorta in cats, only a small proportion of the radioactivity was recovered from the central nervous system.

2. Similar results were obtained when the prostaglandin was given intravenously and intra-arterially to chicks.

3. The results of this study confirm work in other species (Hansson & Samuelsson, 1965 ; Samuelsson, 1964, 1965) and suggest that prostaglandin E_1 exerts its central pharmacological actions in small concentrations.

Pharmacological actions of prostaglandin E_1 (PGE_1) on the central nervous system (CNS) have been demonstrated in cats by injection into the cerebral ventricles (Horton, 1964 ; Horton & Main, 1965), by micro-iontophoretic application (Avanzino, Bradley & Wolstencroft, 1966), by intravenous injection (Horton & Main, 1967) and by close-arterial injection to the spinal cord (Duda, Horton & McPherson, 1968). Although only relatively small doses are required to produce these effects in cats and also on intravenous injection in young chicks (Horton, 1964 ; Horton & Main, 1965, 1967), it has been shown in other species (rats, mice and sheep) that little or no tritium-labelled prostaglandin E_1 reaches the CNS following intravenous injection (Hansson & Samuelsson, 1965 ; Samuelsson, 1964, 1965).

In this investigation, tritiated PGE_1 has been injected into cats and chicks intraventricularly, intra-arterially and intravenously in doses known to produce central nervous effects. The results show that only a small proportion of the injected tritiated PGE_1 is present in the brain or spinal cord when these pharmacological effects are observed.

Methods

Injections into the cerebral ventricles of conscious cats

Collison cannulae were implanted into the lateral ventricles of three cats under pentobarbitone sodium (40 mg/kg) anaesthesia (Feldberg & Sherwood, 1953). After an interval of at least 5 days, injections were made through the cannula without

anaesthesia. PGE_1 was dissolved in 0.9% NaCl solution. Injections were always of 0.2 ml. and were washed in with a further 0.1 ml. of saline solution. After a predetermined time, the animals were anaesthetized with intravenous thiopentone sodium and killed by exsanguination ; during anaesthesia the brain was exposed and immediately after death tissue and fluid samples were taken for assay. The brain was divided into various anatomical regions which were extracted separately (see Table 1).

Injection into the left vertebral artery

A cat was anaesthetized with pentobarbitone sodium (40 mg/kg i.p.). The chest was opened and the animal was artificially ventilated. The left internal mammary artery was cannulated for retrograde injection into the vertebral artery and the left brachial artery was tied off. A thread was placed around the superior vena cava ready for occlusion and both external jugular veins were cannulated for collection of blood from the head. PGE_1 in saline solution was injected slowly into the internal mammary artery, the superior vena cava being occluded throughout from the start of the injection. The animal was exsanguinated through both external jugular veins. The brain was removed immediately after death.

Injection into the left common carotid artery

A similar injection procedure was followed except that the left lingual artery was cannulated for retrograde injection.

Injection into the abdominal aorta

In a third cat under pentobarbitone anaesthesia, the renal, superior mesenteric, inferior mesenteric and splenic blood vessels were ligated. The intestines, pancreas and spleen were removed. The aorta and inferior vena cava were ligated at their distal ends and the left renal artery was cannulated for retrograde injection into the aorta. PGE_1 in saline solution was injected over a period of 1 min into the renal artery ; 2 min after the start of injection the aorta was cannulated and the animal was exsanguinated. The spinal cord ($L1$ to $C1$) and liver were removed immediately after death.

Intravenous injection into chicks

Chicks 2–3 days old weighing 35–45 g were used. Injections were made into the right external jugular vein in a volume of 0.5 ml. The chicks were killed by decapitation, 1–2 min after injection. The brain, liver and remaining carcass were extracted separately.

Intra-arterial injection into chickens

Chickens (800–850 g) were anaesthetized with anaesthetic ether and their common carotid arteries were exposed. Injections of prostaglandin dissolved in 0.25–0.75 ml. of 0.9% NaCl solution were made into the right carotid artery. The chickens were killed by decapitation 30 sec after injection and their brains were removed.

Preparation of samples for assay

Tissue samples were macerated in 96% ethanol and were allowed to stand for 2 hr. The samples were then centrifuged and the supernatant was removed. The sediment was washed again with 96% ethanol and re-centrifuged. The sediment was discarded. The combined supernatants were taken to dryness under reduced pressure at 50° C. The residue was taken up in either 12 ml. of methanol or the minimum quantity permitting complete solution, whichever was the greater. Blood samples were diluted with an equal volume of 0.1 N-HCl and partitioned twice with an equal volume of ethyl acetate (Holmes, Horton & Stewart, 1968). The combined organic phases were taken to dryness and the residue was taken up in 12 ml. of methanol. Samples of cerebrospinal fluid were evaporated to dryness and the residues were dissolved in 12 ml. of methanol.

Estimation of tritium in extracts

This was carried out in a Packard "Tri-carb" liquid scintillation counter using the internal standard technique. Four millilitres of the methanolic tissue extracts were always used with 10 ml. of a scintillator solution of the following composition: 2,5-*bis*-[5′-*t*-butyl-benzoxazolyl (2′)]-thiophene (CIBA), 7 g; toluene, 600 ml.; methycellosolve, 400 ml.; naphthalene, 80 g. All results are expressed as the mean of at least three 10 min counts. The tritiated PGE_1 used in this study had an activity of 138×10^6 d/min/mg.

Artificial cerebrospinal fluid

The artificial cerebrospinal fluid (c.s.f.) was of the following composition: $MgSO_4$, 205 mg; $CaCl_2$, 120 mg; KCl, 210 mg; $NaHCO_3$, 2.3 g; NaCl, 7.38 g; made up to 1 l. with water.

Results

Injections into the lateral cerebral ventricle of unanaesthetized cats

A mixture containing equal parts of tritiated and unlabelled PGE_1 was used in this study. The total dose injected into the lateral ventricle of each unanaesthetized cat was 50 μg PGE_1 contained in 0.2 ml. saline solution.

In the first experiment, where the injection was made into the left lateral ventricle, decreased motor activity was observed 20 min after injection, and after 1 hr there were definite signs of stupor and some catatonia; 105 min after injection, the catatonia and stupor were still increasing. The animal was then anaesthetized and its ventricular system was perfused with 10 ml. of artificial c.s.f., the perfusate being removed through a needle placed in the cisterna magna. The animal was exsanguinated and the brain, liver, gall blaader and kidneys were dissected out. All these tissues were extracted and their tritium content estimated. The results are summarized in Table 1. The largest amount of radioactive PGE_1 was found in the liver. The kidneys and c.s.f. contained appreciable amounts, but the brain contained less than 5% of the injected prostaglandin. This was distributed throughout all the regions (see Table 1). It is of interest to note that when the liver extract was partitioned between an aqueous phase at pH 3 and ethyl acetate, nearly all the radioactivity went into the aqueous phase, which indicated that the tritium was

no longer incorporated into prostaglandin but was in the form of a less acidic or more water-soluble compound which is possibly a metabolite.

In the second experiment a similar procedure was followed but the animal was anaesthetized and exsanguinated 5 min after injection into the left lateral ventricle. No signs of stupor or catatonia developed in that time. Results similar to those of the first experiment were obtained with higher levels in the c.s.f. (Table 1). Even 5 min after injection into the ventricular system, appreciable amounts of radioactivity were recovered from the liver.

In the third cat the injection was made into the right lateral ventricle. Catatonia developed very rapidly and was marked 20 min after injection. After 35 min the animal was anaesthetized and exsanguinated. In this experiment the ventricular system was not perfused but any pools of c.s.f. found during the dissection were removed and each tissue sample was washed with saline solution to remove any activity present in the c.s.f. adhering to the tissue surface, but little activity was present in the washings. Similar results to those in the two preceding experiments were obtained (Table 1).

Intra-arterial injections into anaesthetized cats

In the first experiment, 10 μg of tritiated PGE_1 was injected retrogradely into the left internal mammary artery, enabling it to pass up the left vertebral artery to the brain. Blood samples were taken every minute from the jugular veins until the animal died during the sixth minute after the start of the injection. The brain was removed immediately after death. No area of the brain (forebrain, midbrain, cerebellum, medulla and pons) contained radioactivity equivalent to as much as 0.1 μg prostaglandin, and 25% of the injected activity was found in the blood, the majority coming out in the first 3 min.

When 10 μg of tritiated PGE_1 was injected retrogradely through the left lingual artery similar results were obtained. Approximately 25% of the injected activity was recovered from the blood collected during 5 min exsanguination, and no part of the brain contained activity equivalent to as much as 0.1 μg of PGE_1.

When a similar retrograde injection of 10 μg was made into the left renal artery, which should have enabled the prostaglandin to reach the spinal cord, 10% of

TABLE 1. *Distribution of prostaglandin* E_1 *after the injection of* 50 μg *into the lateral ventricles of cats*

Area	Cat		
	1	2	3
Cerebrum	1·89 (98)	2·92 (132)	<1·0 (<44)
Midbrain	0·10 (100)	0·30 (321)	0·79 (528)
Cerebellum	0·18 (55)	0·70 (194)	1·39 (283)
Medulla and pons	0·25 (125)	0·64 (256)	1·24 (730)
Spinal cord	—	—	≪0·2 (≪27)
Liver and gall bladder	19·42	0·92	3·00
Kidney	1·08	<0·2	1·18
Lungs	—	0·2	≪0·2
c.s.f.	1·19	11·92	0·79
Blood	—	—	0·47
Washings of brain tissue samples	—	—	<0·2

The content of each area is given in μg followed in brackets by the concentration in ng/g.

the injected activity was recovered from the 1.5 min blood samples taken from the aorta. The lumbar and sacral regions of the spinal cord were found to contain activity equivalent to <0.1 μg of PGE_1. About 10% of the injected activity was recovered from the liver, indicating that not all the hepatic blood vessels had been occluded.

Intravenous injection into chicks

Tritiated PGE_1 (2 μg) was injected into each of three chicks, which all showed marked sedation within 90 sec of injection. The chicks were killed at the time of peak effect (1–2 min). The brain of each chick was assayed separately and their livers were pooled for assay, as were the rest of their carcasses. The three brains contained 1.7%, 0.8% and 0.1% respectively of the activity injected. The livers contained 5% and the remaining carcasses 85% of the injected activity.

Intra-arterial injection in chickens

After 1 μg of tritiated PGE_1 was injected into the right common carotid artery of an 800 g chicken, 2% of the injected activity was present in its brain. A similar result was obtained when 3 μg of tritiated prostaglandin was injected into a second chicken. When 5 μg of unlabelled PGE_1 was injected into the right external jugular vein of an unanaesthetized chicken, pharmacological effects (loss of the righting reflex and stupor) similar to those seen in young chicks were observed.

Discussion

The results obtained in this study support Samuelsson's findings in other species that very little radioactivity is found in the central nervous system after intravenous or subcutaneous administration of tritiated PGE_1 (Hansson & Samuelsson, 1965; Samuelsson, 1964, 1965). They further show that even when PGE_1 is injected more directly towards the central nervous system, only small amounts can be detected in the brain and spinal cord. Furthermore, in the young chick, originally selected for studying central effects of prostaglandins because it is said to lack a blood-brain barrier, less than 40 ng of PGE_1 could be detected in the brain following intravenous injections of pharmacologically effective doses.

PGE_1 appears to cross the cerebrospinal fluid-blood barrier, for 5 min after injection into the lateral ventricle, 2% of the injected activity was found in the liver. Whether this is due to uptake through the blood vessels of the choroid plexus or whether the prostaglandin is transported rapidly through the brain tissue and thence to the cerebral capillaries is unknown. From present evidence however, it seems that by whatever route PGE_1 is administered, relatively little is taken up by the central nervous system of either the cat or the chick. The midbrain, medulla and pons consistently contained higher quantities of radioactivity after intraventricular injection than other regions. The uptake by these areas is unlikely to be an artefact resulting from contamination of the tissue with cerebrospinal fluid adhering to the surface, because negligible activity was detected in the washings when the pieces of tissue were washed after dissection.

The dose of PGE_1 employed in each experiment was shown to be sufficient to produce central pharmacological effects. Thus it would seem that only minute amounts of PGE_1 are required to produce these pharmacological effects on the central nervous system.

The prostaglandin used in this study was tritiated on C_5–C_6 and no known metabolite could be present without radioactivity. The possibility that the central effects observed could be due to a peripheral action of PGE_1 modifying afferent impulses to the brain can be discounted, because very much higher doses are required to produce effects when the drug is given intravenously rather than into the lateral ventricle.

The finding that the radioactivity present in the liver of the cat killed in 105 min after intraventricular injection behaved in a different manner from PGE_1 in the HCl-ethyl acetate solvent partition is of interest. All the known immediate metabolites of PGE_1 would behave in a similar manner to the parent compound in this partition system. Thus it would seem that the activity present in the liver is a simpler, less acidic or more water-soluble compound; this confirms the results of Samuelsson (1964) in the rat after subcutaneous administration of labelled PGE_1.

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