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The spontaneous release of prostaglandins into the cerebral ventricles of the dog and the effect of external factors on this release

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Summary

1. Prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ have been identified in perfusates of the cerebral ventricles of anaesthetized dogs.

2. Infusions of serotonin into the lateral ventricle caused a four-fold increase in the release of prostaglandins E into the ventricles and this increase was dissociated from the hyperthermic action. Intraventricular infusions of adrenaline and noradrenaline had no effect on the level of prostaglandin release.

3. Neither electrical stimulation of a hind foot pad nor the intraperitoneal administration of chlorpromazine, amphetamine, tranylcypromine or imipramine had any consistent effect on the amounts of prostaglandins released into the cerebrospinal fluid.

4. When prostaglandin E_1 was added to the fluid perfusing the ventricular system, respiratory changes were observed but almost all the added prostaglandin was recovered from the perfusate leaving the cisterna.

Introduction

The release of prostaglandins and prostaglandin-like substances from the central nervous system of the cat has been reported by several groups of workers: Ramwell & Shaw (1966) reported their release into superfusates of the somatosensory cortex and that afferent stimulation affected the level of release; Feldberg & Myers (1966) demonstrated the release of prostaglandin-like substances from the inferior and anterior horns of the lateral ventricles and from the third ventricle; Wolfe, Coceani & Pace-Asciak (1967) reported their release into superfusates of the cerebellar cortex.

The present work concerns the identification of the prostaglandins released into the cerebral ventricles of the anaesthetized dog under resting conditions and the effect of afferent stimuli and drugs on the level of this release.

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Methods

Perfusion of the cerebral ventricles

Male dogs, 6 to 10 kg, were anaesthetized with intravenous thiopentone sodium followed by intravenous α-chloralose (Roche, 60–80 mg/kg). The trachea and left femoral artery were cannulated and the dog's head was placed in a stereotaxic apparatus (Baltimore Instrument Co. Inc.). The skin, connective tissue and muscle were removed from the top of the skull. A cannula was placed with its tip in the inferior horn of the left lateral ventricle by the following procedure: the cannula was inserted 3 cm in front of the lambda and 1 cm lateral to the saggittal suture to a depth of about 2·4 cm from the surface of the skull; reasonably consistent placement of the cannula tip was obtained. A second cannula was passed through the muscles at the back of the neck and the atlanto-occipital membrane so that the tip was in the cisterna magna. Both cannulae were No. 2 serum hypodermic needles. Artificial cerebrospinal fluid was perfused through the brain from the left lateral ventricle to the cisterna magna using a Watson-Marlow peristaltic pump at a rate of 0·4 to 0·5 ml/min. Negative pressure was not applied to the outflow cannula. The first 15 min perfusate was discarded in all experiments. At the end of each experiment a non-diffusible dye was passed through the perfusion system so that the areas perfused could be verified: these were the left lateral ventricle, the third ventricle, the aqueduct and the fourth ventricle; the right lateral ventricle usually exhibited only faint staining.

Arterial blood pressure was recorded using a Statham P23Db pressure transducer and a Beckman Dynograph. Drugs were administered intraperitoneally in 0·9% saline. When electrical stimulation was used it was provided through wires sewn into the right hind foot pad connected to a Grass model SD5 stimulator.

Artificial cerebrospinal fluid

The artificial cerebrospinal fluid used 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 litre with water.

Extraction and estimation of prostaglandins

Perfusate samples were brought to pH 3 with hydrochloric acid and subjected to the solvent partition, silicic acid column and thin-layer chromatographic and bioassay procedures described by Holmes & Horton (1968) for the identification and estimation of prostaglandins in brain. In some of the earlier experiments thin-layer chromatography using Gréen & Samuelsson's (1964) AI system was employed instead of silicic acid column chromatography to separate the prostaglandins E from the F's. When no difference in biological activity was observed between the control and test samples at the end of the solvent partition system it was considered unnecessary to carry out chromatographic procedures.

Results

Spontaneous release of prostaglandins into the cerebral ventricles

A 6 h perfusion sample of the cerebral ventricles of a dog was collected. The extract of this fluid obtained by the solvent partition procedure contained sub-

stances which contracted the rat fundus preparation. On silicic acid column chromatography two main peaks of biological activity could be separated corresponding in elution volume to prostaglandins E and F. The fractions from these peaks were pooled separately and chromatographed on thin-layer plates of silica gel impregnated with silver nitrate using the AII solvent system of Gréen & Samuelsson (1964). Zones from the preparative plates corresponding to the position of authentic prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ on the master plates were found to contain biological activity. Other zones including those corresponding to prostaglandins E_3 and $F_{3\alpha}$ contained negligible amounts of biological activity.

Further evidence of identification was obtained from parallel biological assays of the material eluted from the thin-layer plates using the appropriate prostaglandin as standard. The results were as follows: prostaglandin E_1, 20 ng on the rat fundus and 40 ng on the guinea-pig ileum ; E_2, 15 ng on the fundus and 20 ng on the ileum ; $F_{1\alpha}$, 75 ng on the rabbit jejunum and 50 ng on the rat uterus ; $F_{2\alpha}$, 10 ng on the jejunum and 25 ng on the uterus. Prostaglandin $F_{1\alpha}$ appeared to be the predominant prostaglandin spontaneously released into the cerebral ventricles of the dog. The total prostaglandin release was of the order of 1 to 5 ng prostaglandin E_1 equiv./min.

Effect of afferent stimuli on the release of prostaglandins

Afferent stimulation caused no consistent effect on prostaglandin release into the cerebral ventricles in the four experiments performed. The afferent stimulation was produced by electrically stimulating the right hind foot pad at a rate of 1 Hz with a pulse width of 10 ms ; the voltage used was sufficient to cause reflex movement of the contralateral limb, indicating that the stimulus at least reached the spinal cord. In one experiment the biological activity of the 67% ethanol residue before chromatography from a 30 min stimulus perfusion showed a fourfold increase over a previous control sample. However, when the residues were further purified by thin-layer chromatography in the AI system it was found that no significant increase in activity of either the prostaglandin E or F zone could be detected in the stimulus sample over the control. In two other experiments using similar stimulus parameters it was found that the prostaglandin release was reduced to 25 to 50% of the control values during hind paw stimulation for 4 h ; this was observed both before and after silicic acid column chromatography. In a fourth experiment no significant difference was noted in the biological activity exhibited by samples from before and during a 1 h stimulus period when they were assayed as the 67% ethanol residues.

TABLE 1. *Effect of amines perfused through the cerebral ventricles upon the release of prostaglandins into the cerebrospinal fluid*

	Prostaglandin release (ng E_1/h)	
Amine	Control	During amine perfusion
Adrenaline 5 μg/ml (275)	65	75
Noradrenaline 20 μg/ml (1,100)	175	180
Serotonin creatinine sulphate 40 μg/ml (2,480)	160	650
Serotonin creatinine sulphate 40 μg/ml (2,720)	140	600
Serotonin creatinine sulphate 40 μg/ml (2,290)	150	600

Values quoted are those of samples before column chromatography in terms of prostaglandin E_1, ng release/h. The figures in parentheses indicate the total quantity of amine infused in μg. Assays were carried out on the rat fundus.

Effect of amines in the cerebrospinal fluid on the release of prostaglandins

The results of these experiments are summarized in Table 1. The perfusion of noradrenaline, 20 μg/ml, and adrenaline, 5 μg/ml, through the cerebral ventricular system for 2 h had no effect on the level of prostaglandin release during the perfusion period.

Serotonin creatinine sulphate, 40 μg/ml, which was equivalent to 20 μg/ml of serotonin base, caused a fourfold increase in the release of prostaglandins E from the ventricles but had no effect on the level of release of the prostaglandins F during a 2 h infusion period. This increase was observed in three experiments: in the first the dog's rectal temperature rose from 37·5° to 41·5° C during the serotonin infusion (2 h) and returned to 37·6° C 1 h after the end of the infusion ; in the other two experiments no hyperthermia was observed but there was a fall in arterial blood pressure. In all these experiments the prostaglandins E were determined as the " E peak " after silicic acid column chromatography.

Effect of drugs administered intraperitoneally on the release of prostaglandins

Neither chlorpromazine hydrochloride (4 mg/kg), amphetamine sulphate (5 mg/kg), tranylcypromine (20 mg/kg) nor imipramine (20 mg/kg) administered intraperitoneally had a consistent effect on the level of prostaglandin release into the cerebrospinal fluid during the 2 h immediately following injection. In all these experiments the dose of drug used was shown to produce pharmacological effects: chlorpromazine caused a sustained fall in blood pressure and a deepening of the level of anaesthesia ; amphetamine caused a near fatal hypertensive crisis during which the outflow from the perfusion system was greatly reduced ; tranylcypromine caused a transient pressor response followed by a sustained gradual rise in blood pressure, presumably due to potentiation of catecholamine actions ; imipramine caused a gradual rise in blood pressure and a deepening of the level of anaesthesia.

In one experiment the administration of chlorpromazine caused a considerable increase in biological activity when the samples were assayed before chromatography. However, after silicic acid column chromatography it was found that the increase in biological activity was due to neither prostaglandins E nor prostaglandins F.

Infusion of prostaglandin E_1 through the cerebral ventricles

In two experiments in which artificial cerebrospinal fluid containing prostaglandin E_1, 100 ng/ml, was perfused through the cerebral ventricular system for 2 h there was a considerable increase in the depth of respiration which lasted until about 10 min after the prostaglandin infusions had stopped. There was no change in arterial pressure. As determined by biological assay on the rat fundus, 22% and −6% of the prostaglandin E_1 were removed from the perfusion fluid during its transit through the ventricular system.

Recovery experiments

When a known amount of prostaglandin E_1 (100 ng/ml) was added to the artificial cerebrospinal fluid, which was then subjected to the solvent partition and silicic acid column chromatographic procedures described in **Methods**, it was found, in two

experiments, that 45–50% of the added activity could be recovered from the appropriate column eluate.

Discussion

The four prostaglandins found in dog brain (Holmes & Horton, 1968) have now been identified in perfusates of the dog cerebral ventricular system. The spontaneous release of each of these prostaglandins is of such a low level that it is very difficult to detect small changes in release with the extraction and assay techniques at present available.

The high level of biological activity before column chromatography in the post-drug sample after the administration of chlorpromazine indicates the danger of making estimates of prostaglandin content from relatively impure extracts. No positive conclusions on changes in levels of prostaglandin release can be made unless the extraction procedure is carried through to, at least, silicic acid column chromatography.

The only external factor tested which consistently affected the release of prostaglandins from the ventricular system was the intraventricular infusion of serotonin. This caused a fourfold increase in the release of prostaglandins E and this appeared to be dissociated from the hyperthermic action of serotonin as it occurred both in the experiment where hyperthermia was observed and in the two experiments where it was not.

Ramwell and co-workers reported that hind paw stimulation, infused serotonin and tranylcypromine but not adrenaline increased the release of prostaglandins from the frog perfused spinal cord (Ramwell, Shaw & Jessup, 1966). They also reported that the release of prostaglandins from the cat somatosensory cortex was increased by paw stimulation (Ramwell & Shaw, 1966). Their observations with serotonin and adrenaline on the cerebral cortex were duplicated for release into the dog's cerebral ventricles, but no increased release was seen after hind paw stimulation. Similar results to those of Ramwell *et al.* might have been obtained from tranylcypromine if it had been perfused through the ventricles instead of being administered intraperitoneally. All the experiments in this study were performed in anaesthetized dogs whereas Ramwell and co-workers used unanaesthetized preparations. The presence of an anaesthetic might account for some of the different results obtained.

The decrease in perfusion outflow observed after the administration of amphetamine is of interest. It was almost certainly not due to a blockage in the perfusion system and may have been caused by vasoconstriction of the pial blood vessels which could lead to an increase in the cerebrospinal fluid space.

No reason can be given for the increased release into the cerebral ventricles of prostaglandins E induced by infused serotonin, but it is of interest that Feldberg & Myers (1966) reported the spontaneous release of both serotonin and a prostaglandin-like substance into the cerebral ventricles of the cat. After the injection of either adrenaline or serotonin into the lateral cerebral ventricle of unanaesthetized cats a listless condition was observed (Feldberg & Sherwood, 1954); a state of catatonic stupor occurs after the intraventricular injection of prostaglandins E_1, E_2 or E_3 (Horton, 1964). The possible interaction of serotonin and the prostaglandins in the central nervous system warrants further study.

In this work net release of prostaglandins into the cerebral ventricular system was studied and no account was taken of possible reabsorption from the third and fourth ventricles and the choroid plexus. However, experiments on the uptake of infused prostaglandin E_1 indicate that very little exogenous prostaglandin is taken up from the cerebrospinal fluid. Further studies should be carried out perfusing restricted areas of the ventricular system.

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REFERENCES

FELDBERG, W. & MYERS, R. D. (1966). Appearance of 5-hydroxytryptamine and an unidentified pharmacologically active lipid acid in effluent from perfused cerebral ventricles. *J. Physiol., Lond.*, **184**, 837–855.

FELDBERG, W. & SHERWOOD, S. L. (1954). Injections of drugs into the lateral ventricle of the cat. *J. Physiol., Lond.*, **123**, 148–167.

GRÉEN, K. & SAMUELSSON, B. (1964). Thin layer chromatography of prostaglandins. *J. lipid Res.*, **5**, 117–120.

HOLMES, S. W. & HORTON, E. W. (1968). The identification of four prostaglandins in dog brain and their regional distribution in the central nervous system. *J. Physiol., Lond.*, **195**, 731–741.

HORTON, E. W. (1964). Actions of prostaglandins E_1, E_2 and E_3 on the central nervous system. *Br. J. Pharmac. Chemother.*, **22**, 189–192.

RAMWELL, P. W. & SHAW, J. E. (1966). Spontaneous and evoked release of prostaglandins from the cerebral cortex of anaesthetized cats. *Am. J. Physiol.*, **211**, 125–134.

RAMWELL, P. W., SHAW, J. E. & JESSUP, R. (1966). Spontaneous and evoked release of protaglandins from frog spinal cord. *Am. J. Physiol.*, **211**, 998–1004.

WOLFE, L. S., COCEANI, F. & PACE-ASCIAK, C. (1967). Brain prostaglandins and studies of the action of prostaglandins on the isolated rat stomach. In *Prostaglandins*, Nobel Symposium 2, ed. Bergstrom, S. and Samuelsson, B., pp. 215–276. Stockholm: Almqvist and Wiksell.

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