PROSTAGLANDINS IN BRAIN AND THE RELEASE OF PROSTAGLANDIN-LIKE COMPOUNDS FROM THE CAT CEREBELLAR CORTEX

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Abstract

A water-soluble lipid material resistant to acid and alkali treatment was found in perfusates and extracts of brain tissue, which induced contraction of the 'slow type' on the isolated rat stomach fundus. The material isolated from brain tissue extracts behaved in solvent partition systems like the prostaglandin compounds and could be purified by silicic acid and thin-layer chromatography. The smooth-muscle-stimulating activity is likely due to the trihydroxyprostaglandin compounds.

The cerebellar cortex contains little acetylcholine (1) and this has been interpreted by most workers as indicating a high density of noncholinergic synapses. The possibility that 5-hydroxytryptamine and histamine may be synaptically functional has been examined previously with inconclusive results (2, 3). During an investigation of the smooth-muscle-stimulating activity of perfusates of the cat cerebellum in vivo the presence of acid- and alkali-stable water-soluble lipid substances was detected. These substances behaved in solvent partition systems and on silicic acid columns like the prostaglandins of U. S. von Euler, chemically characterized recently by Bergström and co-workers (4, 5).

Methods and Results

Cats were decerebrated by section of the brainstem at the intercollicular level. The surface of the anterior lobe of the cerebellum was exposed and perfused continuously with Elliott solution (6) at 37 °C delivered through a calibrated infusion set at a rate of 1.3–1.5 ml/15 minutes. Surface and unit electrical activity was monitored. The smooth-muscle-stimulating activity of samples of the perfusate was tested on either the rat stomach fundus strip preparation of Vane (7) or on the guinea pig ileum.

No activity was found on the guinea pig ileum which could be accounted for as histamine, even when the tissue was sensitive to 1.5×10^{-9} g/ml (1.5 ng/ml) histamine base. The perfusates contained 5-hydroxytryptamine as determined on an atropinized stomach fundus strip. The activity due to 5-hydroxytryptamine was completely destroyed by treating the samples at 100° for 3 minutes at pH 10–11. The presence of 5-hydroxytryptamine was further confirmed by testing some of the samples on the muscle treated with methysergide bimaleate (Sansert). The amount released varied greatly (0.04–3.3

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ng/minute cm² cerebellar surface), and no obvious correlation was found with the level of electrical activity, nor could the minimal amounts of blood present in the perfusate account for the variations in output. Also the variation could not be related to interference by catecholamines.

It was observed that the perfusates contained an alkali-stable principle active on the rat fundus strip in the presence of atropine and the 5-hydroxytryptamine antagonists, 2-bromolysergic acid diethylamide (BOL-148) or methysergide bimaleate. The contraction induced by this principle was of the 'slow' type, that is, it started after a 15–20 second delay, failed to reach the peak within 90 seconds, and was always followed by a period of increased tone. The activity was higher when neostigmine was absent from the perfusion fluid. No loss of activity occurred after the samples were heated to 100° for 3 minutes in alkali (pH 10) or acid (pH 2). The amount of alkali-stable principle determined in perfusates without neostigmine, when expressed in terms of acetylcholine (ACh), was equivalent to 2–8 ng ACh/15 minutes cm² of cerebellar surface. Thus the perfusates contained a smooth-muscle-stimulating substance whose properties differed from those of 5-hydroxytryptamine, catecholamines, acetylcholine, substance P, the cerebellar factor of Crossland, or ergothioneine (8).

Slabs of cerebral and cerebellar cortex were dissected quickly from the exposed brain of cats anaesthetized with ether, and the tissue was frozen in liquid nitrogen. The tissue was extracted with 5% trichloracetic acid (TCA) while thawing. The TCA was removed by repeated extractions with small volumes of ether. An alkali-stable smooth-muscle-stimulating substance was found in the extracts both from cerebral and cerebellar cortex, whose activity on an atropinized and methysergide bimaleate-treated muscle was of the same type as that found in the perfusates. The active substance was greatly diminished in TCA extracts from cerebral cortex homogenates which had been incubated for 1 hour at 37°, and the activity was reduced in amount if the tissue was allowed to thaw before TCA was added. We considered that we might be dealing with an acidic phospholipide such as acetal phosphatidic acid or Darmstoff (9), but this seemed unlikely since the activity of perfusates and extracts was not diminished after they were boiled in acid at pH 1.5 in a sealed tube for 20 minutes. Substance P or other polypeptides were excluded because the type of contraction induced was unaffected when the samples were treated with crystalline chymotrypsin at pH 8.5 (10). The alkali-stable material did not partition readily into ether from an acidified homogenate of brain tissue. Therefore, it was considered unlikely that the smooth-muscle-stimulating principle was one of the hydroxy fatty acids reported by Ambache and coworkers (11).

The prostaglandins described by U. S. von Euler (4) in crude extracts of seminal vesicles and fluid of man and sheep stimulate isolated intestine, are stable to short periods of acid and alkali treatment, and have a low ether partition coefficient. The possibility was tested that this type of compound might account for the alkali-stable smooth-muscle-stimulating activity in the

perfusates and TCA extracts of brain. Prostaglandins have been recently chemically characterized as a group of hydroxy-keto C_{20} unsaturated fatty acids (5). A cat was anaesthetized with ether and the total forebrain (20 g) was removed and frozen immediately in liquid nitrogen, and the prostaglandins were extracted by the procedure outlined by Eliasson (12). In summary, the brain was homogenized from the frozen state in 2 volumes of acid–acetone, the suspension was filtered, and the filtrate concentrated *in vacuo*, acidified to pH 3.0, and partitioned with ethyl acetate. The ethyl acetate phase was washed with 0.1 *M* phosphate buffer, pH 7.8, and the aqueous phase acidified to pH 3.0 and extracted again with ethyl acetate. The ethyl acetate phase was evaporated *in vacuo* to dryness and the residue partitioned between equal volumes of petroleum ether and ethanol–H_2O (2:1) and the aqueous phase concentrated. The concentrate showed smooth-muscle-stimulating properties similar to those found in alkali-treated perfusates and TCA extracts (Fig. 1A). Since we did not have pure standards of the different types of prostaglandins, one unit of activity was arbitrarily defined as that present in an aliquot of concentrate inducing a contraction equivalent to that produced by 30 ng of acetylcholine. For the cat brain a unit corresponded to 900 mg wet weight of tissue.

The same procedure as outlined above was repeated with 200 g of fresh frozen ox brain, and the dried aqueous extract dissolved in ethyl acetate – benzene (30:70) and fractionated on a silicic acid column (Biorad) according to the procedure of Samuelsson (13). The results are shown in Fig. 2. The major peak of activity occurred in the ethyl acetate – benzene (80:20) eluates which have been shown by Samuelsson to contain the prostaglandin F compounds (the trihydroxy fatty acids). The type of activity on the muscle was similar to that previously encountered (Fig. 1B). Table I shows the purification achieved during this fractionation procedure. To achieve further purification the ethyl acetate (80:20) eluate from the silicic acid column was concentrated to a small

TABLE I

Purification of prostaglandins from ox brain

Source	Units*/g solids
Ethyl acetate partition from acid–acetone extract	650
Aqueous phase from petroleum ether – ethanol – water partition	3,300
Silicic acid chromatography	
Ethyl acetate – benzene 60:40	0
Ethyl acetate – benzene 80:20	21,400
Ethyl acetate	—
Methanol	0
Thin-layer chromatography of band R_f 0.39–0.48	700,000

*One unit = the activity on the rat fundus strip preparation of 0.05 ml of the initial extract, equivalent in activity to 30 ng acetylcholine chloride. The 5-hydroxytryptamine activity was blocked with methysergide bimaleate 2×10^{-7} g/ml.

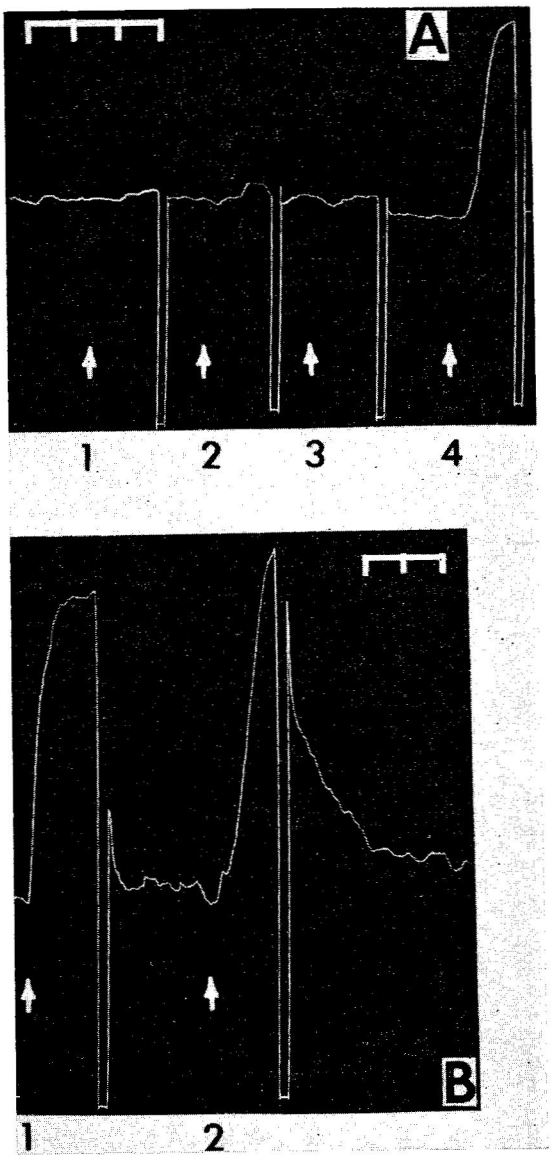

FIG. 1A. Activity on the isolated rat stomach fundus strip of the concentrated aqueous ethanol phase of an extract from cat brain following partition from petroleum ether (see text). Bath volume, 9 ml; Tyrode perfusion fluid containing 2×10^{-7} g/ml methysergide bimaleate and 1×10^{-7} g/ml atropine at 37 °C; time in minutes. (1) 50 ng serotonin creatinine $H_2SO_4.5H_2O$; (2 and 3) 50 ng acetylcholine chloride; (4) 0.05 ml brain extract.

FIG. 1B. The type of contractions exerted on the isolated rat stomach fundus by acetylcholine and the ethyl acetate (80:20) eluate from a silicic acid column of extracts of ox brain. Tyrode perfusion fluid. (1) 30 ng acetylcholine chloride; (2) silicic acid column eluate.

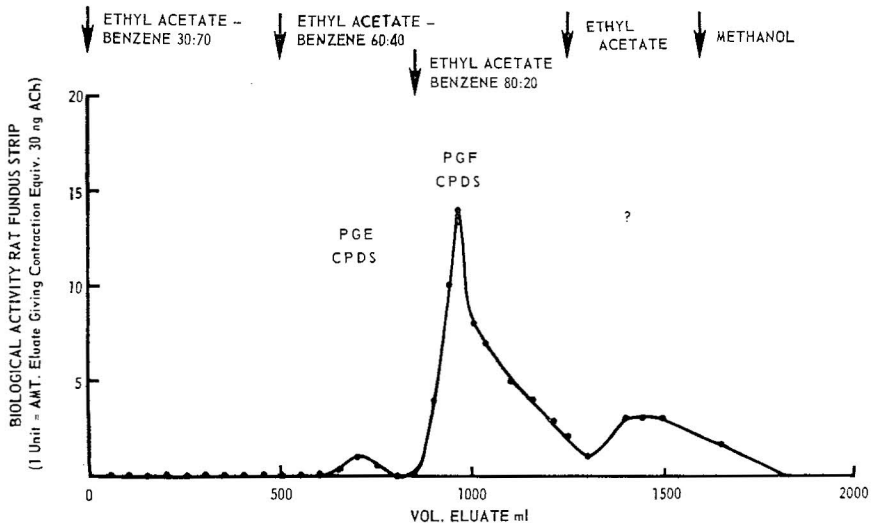

FIG. 2. Separation of prostaglandins in an aqueous ethanol extract from ox brain (200 g) by silicic acid chromatography (see text). Column, 50 g; fractions, 40–50 ml; biological activity assayed in a 9 ml bath on the isolated rat stomach fundus at 37°; Tyrode solution containing 2×10^{-7} g/ml methysergide bimaleate.

volume and applied to thin-layer plates of silica gel without binder and developed in benzene–dioxane (5:4) (14). The smooth-muscle-stimulating activity was found only in a band of R_f 0.39–0.48 (see Table I). Elution of the material from this band with methanol, methylation with diazomethane, and rechromatographing on thin layers in benzene–dioxane (5:4) resulted in smooth-muscle-stimulating activity only in extracts of a band of R_f 0.20–0.28. Since the amount of material we obtained after elution from the thin layers was very small, it was not possible to establish whether the prostaglandins present were of the $PGF_{3\alpha}$ or of the $PGF_{2\alpha}$ types.* However, it seems most likely that prostaglandins of the PGF type are present in our cerebellar perfusates, TCA extracts, and acid–acetone extracts and account for the alkali-stable smooth-muscle-stimulating activity.

Comment

The extractability of prostaglandins from brain tissue differed from that described by Samuelsson (13) for extraction from human seminal fluid. We could not obtain biological activity from acid–ethanol extracts of brain tissue subsequently partitioned with ether but only with ethyl acetate. However, the active material obtained from the silicic acid column did indeed partition into ether. The smooth-muscle-stimulating substance considered in our extracts to be due to a prostaglandin is almost certainly the same material as the unidentified substance which stimulated smooth muscle reported in TCA and acetone extracts of cerebral hemispheres by Andrews, Price, and West (16).

*Recently Samuelsson (15) fully characterized chemically the major prostaglandin of bovine brain as of the '$PGF_{2\alpha}$' type.

NOTE ADDED IN PROOF: Since this paper was submitted, Dr. S. Bergström, Karolinska Institute, Sweden, generously supplied us with crystalline prostaglandin E_1 (PGE_1) for a standard. We found that 6 ng of crystalline PGE_1 induced a contraction on the non-atropinized rat fundus strip equivalent in amplitude to 30 ng ACh (one unit in this paper). The threshold of sensitivity was 1–2 ng PGE_1 added to a 9 ml bath. The type of contraction induced by crystalline PGE_1 was identical with that found in the cerebellar perfusates and in the active fractions obtained after chromatography.

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References

1. F. C. MACINTOSH. J. Physiol. (London) **134**, 296 (1941).
2. J. CROSSLAND. *In* Metabolism of the nervous system. *Edited* by D. Richter. Pergamon Press, London. 1960. p. 523.
3. F. COCEANI, E. FADIGA, and T. GESSI. Boll. Soc. Ital. Biol. Sper. **38**, 1624 (1962).
4. U. S. VON EULER. J. Physiol. (London) **88**, 213 (1937).
5. S. BERGSTRÖM, R. RYHAGE, B. SAMUELSSON, and J. SJÖVALL. J. Biol. Chem. **238**, 3555 (1963).
6. K. A. C. ELLIOTT and H. H. JASPER. J. Neurosurg. **6**, 140 (1949).
7. J. R. VANE. Brit. J. Pharmacol. **12**, 344 (1957).
8. J. CROSSLAND, G. N. WOODRUFF, and J. F. MITCHELL. Nature, **203**, 1388 (1964).
9. W. VOGT. J. Physiol. (London) **137**, 154 (1957).
10. B. PERNOW. Acta Physiol. Scand. **34**, 295 (1955).
11. N. AMBACHE, M. REYNOLDS, and J. M. C. WHITING. J. Physiol. (London) **166**, 251 (1963).
12. R. ELIASSON. Acta Physiol. Scand. **46**, Suppl. 158 (1959).
13. B. SAMUELSSON. J. Biol. Chem. **238**, 3229 (1963).
14. K. GREEN and B. SAMUELSSON. J. Lipid Res. **5**, 117 (1964).
15. B. SAMUELSSON. Biochim. Biophys. Acta, **84**, 218 (1964).
16. P. L. F. ANDREWS, S. A. P. PRICE, and G. B. WEST. Nature, **187**, 420 (1960).