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Correlation of prostaglandin release from the cerebral cortex of cats with the electrocorticogram, following stimulation of the reticular formation

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1. Prostaglandin-like material has been found in superfusates of cerebral cortex in unanaesthetized *encéphale isolé* cat preparations.

2. The material was assayed on the isolated rat uterus and identified by thin-layer chromatography.

3. The level of spontaneous release of prostaglandin-like material was greater than that which had been found in anaesthetized preparations and it increased further with electrical stimulation of the reticular formation which induced electrocortical arousal.

4. Chlorpromazine (1·0–8·0 mg/kg) not only depressed the spontaneous release but blocked the increase evoked by stimulation concomitantly with blocking electrocortical arousal. Increasing the stimulating voltage to restore the arousal response also restored the evoked release of prostaglandins.

5. Most of the prostaglandin-like material released spontaneously was represented by E type compounds, but the increase with stimulation was mainly of F compounds.

The prostaglandins are widely distributed in mammalian tissues (Bergström, Carlson & Weeks, 1968), including the central nervous system of various species (Coceani & Wolfe, 1965; Horton & Main, 1967a; Samuelsson, 1964); they have also been shown to have actions on the brain when injected intraventricularly (Horton, 1964) or when applied iontophoretically to single neurones (Avanzino, Bradley & Wolstencroft, 1966). The spontaneous release of prostaglandins into superfusates of cat somatosensory cortex, demonstrated by Ramwell & Shaw (1966), was increased by electrical stimulation of the contralateral cortex and contralateral superficial radial nerve. The effect was found to be frequency dependent, suggesting that nervous pathways might be involved.

Thus, there is evidence that the prostaglandins may be implicated in some way with neuronal activity in the central nervous system and the detection of these sub-

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stances in subcellular particles derived from nerve endings (Kataoka, Ramwell & Jessup, 1967) supports this view.

The present investigation was undertaken to determine whether there was any correlation between the release of prostaglandins from the cerebral cortex and the pattern of activity in the electrocorticogram. The experiments of Ramwell & Shaw (1966) were carried out on anaesthetized preparations and it is not known whether the release of prostaglandins was influenced by the presence of anaesthetic. Since these agents also modify the electrical activity of the brain, the experiments reported here were all carried out on unanaesthetized *encéphale isolé* preparations. Changes in the electrocorticogram were produced by electrical stimulation of the brain stem reticular formation or by administration of drugs.

Methods

Experiments were performed on a total of fifteen adult cats. Anaesthesia was induced with ethyl chloride and maintained with halothane (1–2%), following intubation. The femoral vein was cannulated for drug and glucose administration, and the head of the cat was clamped in a stereotaxic instrument. The *encéphale isolé* preparation was made by sectioning the spinal cord at the C1 level and artificial respiration then initiated (see Bradley & Elkes 1957 for details).

Six cortical recording electrodes (Bradley & Elkes, 1953) were placed over the right hemisphere and a bipolar concentric stimulating electrode (Bradley & Key, 1958) was stereotaxically inserted into the midbrain reticular activating system (Horsley-Clarke coordinates F+6, T+1·5, H−2). After the position of the tip had been verified (by applying a stimulus to the electrode and observing electrocortical arousal), the electrode was secured to the skull with dental wax. Electrocortical activity was monitored continuously.

A small area of the left cerebral cortex (approximately 1 cm^2) was prepared for superfusion as previously described (Ramwell & Shaw, 1964). All wound edges were infiltrated with local anaesthetic (procaine), the cat was removed from the stereotaxic instrument and halothane was withdrawn. After 1 hr. consecutive samples of superfusate (1·5 ml./10 min) were collected before, during and after electrical stimulation of the brain stem reticular formation, which was carried out for a period of 10 sec every minute for 10 min. The threshold for eliciting electrocortical arousal was determined by progressively increasing the stimulating voltage using square wave pulses at 300/sec, 0·1 msec duration (Bradley & Key, 1958). Blood pressure was recorded from the femoral artery in some experiments.

Bioassay preparation

The isolated uterus from a non-oestrous rat was suspended in a 0·5 ml. Perspex bath containing aerated de Jalon's solution at 22° C. Contractions were recorded isometrically with a strain gauge transducer on an ink polygraph in the presence of BOL (2-bromolysergic acid diethylamide, 0·25 μg/ml.) and atropine (0·15 μg/ml.). The specificity of this preparation and factors governing its response have been studied in detail (Ramwell & Shaw, 1966).

The smooth muscle stimulating activity of aliquots of 10 min samples of the cortical superfusate was determined using PGE_1 as a standard and compared with

the activity of diethyl ether extracts of acidified (pH 2·5–3) aliquots. The shapes of the log dose response curves for PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ on this preparation were found not to deviate significantly.

Thin-layer chromatography

The smooth muscle stimulating material of ether extracts of the acidified superfusate, was developed on 0·25 mm layers of silica gel G in the AI solvent system to separate the groups of prostaglandins, followed by the AII solvent system (Gréen & Samuelsson, 1964) to separate the individual prostaglandins (Shaw, Ramwell & Plasse, 1968). The R_F of the pharmacologically active material was located by bioassay of 50% methanol eluates of areas of the silica gel. PGE_1, PGA_1, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were concurrently developed as standards, and detected by spraying with 10% phosphomolybdic acid in ethanol and heating to 110° C for 5–10 min.

Inactivation procedures

The pharmacologically active material remaining in the acidified cortical superfusate after ether extraction was subjected to various pharmacological inactivation procedures including chymotrypsin digestion, and treatment with phenyl isocyanate, thioglycollate and carboxypeptidase (Shaw & Ramwell, 1969).

Drugs

Chlorpromazine hydrochloride (1·0–8 mg/kg) dissolved in 1 M NaCl) and pentobarbitone sodium were administered intravenously. Prostaglandin standards were supplied by Dr. J. E. Pike, Upjohn Company, Michigan, U.S.A.

Results

Spontaneous and evoked release

A material which stimulated the isolated rat uterus was released into superfusates of the cerebral cortex of the *encéphale isolé* preparation. The response of the bioassay preparation was not modified by the presence of choline ester or alkyl indole antagonists. In eight experiments the release was equivalent to $1{\cdot}53 \pm 0{\cdot}25$ ng PGE_1/cm^2 per min. Most of the active material partitioned into diethyl ether at pH 2·5, suggesting an association with the prostaglandins. The amount of ether-soluble material released was equivalent to $1{\cdot}18 \pm 0{\cdot}25$ ng PGE/cm^2 per min (Table 1). In one experiment, in which the spinal cord was not sectioned, and the animal was anaesthetized with pentobarbitone (25 mg/kg intravenously), the basal release of pharmacologically active material was lower than in any of the *encéphale isolé* preparations and was equivalent to 0·55 ng PGE_1/cm^2 per min. Induction of anaesthesia with pentobarbitone in an encéphale isolé preparation was found to reduce the spontaneous release of prostaglandin-like material by 25%.

In six *encéphale isolé* preparations electrical stimulation (2–5 v) of the mesencephalic reticular activating system elicited electrocortical arousal, and increased by 75–200% the release of the uterus stimulating material from cortex; more than 90% of this material partitioned into diethyl ether at pH 2·5–3.

Effect of chlorpromazine

Within 20–30 min of injection, chlorpromazine (1·0–2·5 mg/kg intravenously) reduced the spontaneous release of acid ether-soluble material from the cortex to the equivalent of $0{\cdot}86 \pm 0{\cdot}27$ ng PGE_1/cm^2 per min, in six animals. In two experiments, the release of pharmacologically active material was decreased by a further 45 and 50% following a second injection of chlorpromazine (8 mg/kg intravenously). It was found that chlorpromazine not only reduced the spontaneous release of prostaglandin-like material, but also blocked the increased release evoked by stimulation of the brain stem (Table 1). At the same time, electrocortical arousal was blocked due to the elevation of the arousal threshold produced by chlorpromazine (Bradley & Key, 1958). The arousal response could be restored by increasing the stimulating voltage by a factor of 2–4, and in three experiments in which this was done the increased release of prostaglandin-like material also reappeared.

Identification

In a separate series of experiments, using five cats, samples of superfusate were collected and pooled before (71 ml.) and during (52 ml.) suprathreshold stimulation; the two samples were acidified, extracted with diethyl ether and estimated by bioassay to contain the equivalent of 2·5 and 4·5 μg PGE_1. Both extracts separated on thin-layer chromatography in the AI solvent system into three peaks corresponding to the R_F values obtained for the PGE, PGF and PGA standards which were simultaneously developed. Most of the active material within the sample collected during stimulation was localized within the PGF zone (Table 2). Since the PGE compounds are readily transformed by acid treatment to the PGA compounds (Hamberg & Samuelsson, 1967), the active material within the PGA zone may represent either transformed PGE compounds or, alternatively, endogenous PGA compounds. The recoveries of active material from the chromatogram (69% and 62%) were of the same order as those observed using $1^{-14}C$ PGE_1 (Shaw & Ramwell, 1969). Further thin-layer chromatography in the AII system of silica gel G con-

TABLE 1

Cat No.	Spontaneous release PGE_1=ng/cm² per min	Voltage*	% increase on stimulation	Spontaneous release 20–30 min after CPZ	Voltage	% increase on stimulation
1	1·26	2	100	—	—	—
3	0·5	2	110	0·5†	2	0
					4	265
6	0·05	2·5	100	0·05†	2·5	0
					10·0	0
10	1·5	3	75	0·75†	3	0
12	1·1	—	—	—	—	—
13	1·1	5	200	0·75†	8	45
					12	230
				0·375‡	20	0
14	1·5	3·5	0	1·1†	6	0
				1·1‡	—	—
15	2·4	0·5	100	2·0†	0·5	0
					0·75	0
				0·9‡	1·5	50

Mean ± S.E.
Control $1{\cdot}18 \pm 0{\cdot}25$ ng $PGE_1 \equiv cm^2$ per min
After CPZ $0{\cdot}86 \pm 0{\cdot}27$
Paired t test: $0{\cdot}05 > P > 0{\cdot}02$

* In each instance the voltage used was sufficient to induce electrocortical arousal.
† 1–2·5 mg/kg chlorpromazine (CPZ).
‡ 8·0 mg/kg CPZ.

taining silver nitrate indicated that the activity within eluates of the PGE and PGF zones of the AI chromatogram could be identified with PGE_1, PGE_2, $PGE_{1\alpha}$ and $PGF_{2\alpha}$ (Table 2). Little difference between the PGE_1 and PGE_2 content of the control and stimulated samples were detected. However, during stimulation of the reticular formation, a marked increase in the release of $PGF_{1\alpha}$ was evident.

The small amount (<10%) of uterus-stimulating material which remained in the acid aqueous phase following partition with diethyl ether was inactivated on incubation with chymotrypsin, thioglycollate, and phenyl isocyanate but was not affected by carboxypeptidase; this indicates that the material may be similar to the substance P polypeptide previously identified in superfusates of the cat cerebral cortex (Shaw & Ramwell, 1968).

Discussion

These experiments provide further support for the hypothesis that there is a direct relationship between the release of prostaglandins from the cerebral cortex of the cat and neuronal activity. Prostaglandin release in the *encéphale isolé* ($1{\cdot}18 \pm 0{\cdot}25$ ng PGE_1 equivalents/cm^2 per min) was greater than that found in anaesthetized animals (0·05 – 0·5, Ramwell & Shaw, 1967) and was still further increased by stimulation of the brain stem reticular formation. A similar increase in prostaglandin release has been found in *encéphale isolé* preparations following injection of picrotoxin (Shaw, unpublished observations). This correlation between the electrocorticogram and prostaglandin release was maintained after administration of chlorpromazine, which blocked the arousal response (for stimulation at threshold levels) and the increase in prostaglandin release.

Although $PGF_{2\alpha}$ (0·3 μg/g) has been definitely identified in bovine brain by mass spectrometry (Samuelsson 1964), the identification and estimation of prostaglandins and prostaglandin-like substances within the central nervous system of dog, cat, and chick brain (Horton & Main 1967a; Holmes & Horton 1968a), as well as superfusates of cerebral cortex (Ramwell & Shaw 1966), cerebellum (Coceani & Wolfe 1965) and cerebroventricular system of the cat (Feldberg & Myers 1966), is tentative, and has depended upon the use of bioassay and thin-layer chromatography procedures similar to those described here.

TABLE 2. *Separation of the PGE, PGF and PGA compounds within superfusates obtained during control periods and electrical stimulation of the midbrain reticular formation on*

	(i) AI chromatogram			
	PGA	PGE	Blank	PGF
R_F prostaglandin standards	0·9	0·83		0·63
		ng PGE_1 equivalents		
Control sample	500	625•	100	500×
Stimulated sample	750	500†	100	1500*
	(ii) AII chromatogram			
	PGE_1	PGE_2	$PGF_{1\alpha}$	$PGF_{2\alpha}$
R_F prostaglandin standards	0·72	0·61	0·56	0·40
		ng prostaglandin equivalents		
Control				
PGE area from AI•	37·5	200	—	—
PGF area from AI×	—	—	150	250
Stimulated				
PGE area from AI†	50	175	—	—
PGF area from AI*	—	—	875	250

The symbols represent the specific areas of the AI chromatogram.

Detection of an increased release of $PGF_{1\alpha}$ during stimulation of the brain stem was similar to the increase in $PGF_{1\alpha}$ previously noted in perfusates of the frog spinal cord following bilateral stimulation of the hind limbs (Ramwell, Shaw & Jessup 1966). Most of the prostaglandins in the supernatant of homogenates of rat cerebral cortex is of the PGF type, and more than 60% of the PGF material in the total homogenate cochromatographed with $PGF_{1\alpha}$ (Kataoka *et al.*, 1967).

The subcellular localization of prostaglandins within noncholinergic nerve endings of rat cerebral cortex, their ready release on nervous and hormonal stimulation, and their known effects following iontophoretic application to single neurones in the brain (Avanzino *et al.*, 1966), all suggest that the prostaglandins fulfil a role related to neurohumoral transmission in the central nervous system. However, an increased release of postaglandins can be detected for as long as 20–30 min following cessation of stimulation (Ramwell & Shaw 1966), and the effects of intraventricular PGE administration to cats can still be detected 48 hr after injection (Horton 1964). Similarly, PGE_1, $PGF_{1\alpha}$, and $PGF_{2\alpha}$ have been found to produce long-lasting depolarization (up to 20 min) of the dorsal and ventral roots of the isolated hemisected spinal cord of the toad, in contrast to the depolarization elicited by glutamate, which returned to control levels within 1 min (Phillis & Tebecis, 1968). Furthermore, the excitatory and inhibitory effects of PGE_1, PGE_2 or $PGF_{2\alpha}$ on brain stem neurones was followed by a unique desensitization, specific for the compound applied (Avanzino *et al.*, 1966). The PGF compounds have a strychnine-like action on the spinal cord of the chick (Horton & Main, 1967b) and on intra-arterial injection close to the spinal cord they produce a reduction in the monosynaptic reflex which is slow in onset and lasts for up to 3 hr (Duda, Horton & McPherson, 1968). These findings, together with the fact that prostaglandins can protect mice against death from electroshock (Holmes & Horton, 1968b), may also be explained in terms of a long-lasting depolarization, which would suggest that the prostaglandins modify neuronal activity by an action on the cell membrane. While it seems unlikely that the prostaglandins are themselves synaptic transmitters in the brain, they may play an important role in modifying the actions of other transmitters, although no correlation was observed between the actions of prostaglandins and those of acetylcholine when applied to the same neurone (Avanzino *et al.*, 1966).

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