J. Pharm. Pharmac., 1973, **25**, 745

LETTERS TO THE EDITOR

The effect of local anaesthetics on the incorporation of ^{32}P into the phosphoinositides of rabbit vagus nerve

Recently, we reported that the local anaesthetic cinchocaine caused a greater than three-fold increase in the incorporation of [^{32}P]orthophosphate into the monophosphoinositides (MPI) of isolated rabbit vagus nerve (Salway & Hughes, 1972). Cinchocaine has now also been shown to increase labelling of MPI by [^{32}P]orthophosphate in rat parotid gland (Lapetina & Michell, 1973) and of both MPI and phosphatidylglycerol in rat pineal gland (Eichberg, Shein & Hauser, 1973). These latter workers suggested that the common factor among several pharmacological agents which they demonstrated to be capable of increasing MPI labelling was the possession of local anaesthetic or "membrane stabilising" properties. Our earlier studies have therefore been extended to see if a variety of other local anaesthetics would increase labelling of MPI by ^{32}P.

The materials used and the preparation and treatment of the vagus nerves were as described by Salway & Hughes (1972) except that the organ bath was modified to have a total of 14 electrodes in two parallel lines. Briefly, desheathed vagi were mounted on the electrodes and allowed to equilibrate for 5 min in 5 ml of physiological saline (NaCl 136, KCl 5·6, $CaCl_2$ 2·2, $MgCl_2$ 1·2, NaH_2PO_4 1·2, $NaHCO_3$ 16·2, glucose 5·5 mM) at 37° and gassed with 5% carbon dioxide in oxygen. Maximal compound action potentials were elicited with rectilinear electrical pulses (0·2 ms duration, 20 V) and the preparations were then treated for 10 min with either amethocaine (0·16 mM), lignocaine (1·42 mM), tropacocaine (2·39 mM) or procaine (2·82 mM). For control nerves an equivalent volume of 0·9% NaCl was added to the organ bath. After this treatment, electrical stimulation was again applied to confirm complete abolition of the induced action potentials in the local anaesthetic treated nerves. Approximately 0·5 mCi ^{32}P (as orthophosphate, 2·56 Ci mol^{-6}, Radiochemical Centre, Amersham) was added to the organ bath and the nerves were incubated for 60 min without further stimulation. Nerves were then stimulated (2 min at 3 Hz), weighed and stored at −20°. (Electrical stimulation was applied to check for viability in the case of the control nerves and the local anaesthetic treated nerves were stimulated similarly.) Extraction and separation of the phospholipids was carried out by paper chromatography and after development, the formaldehyde treated papers were sprayed with 0·25% (w/v) ninhydrin in acetone and were heated in an oven at 100° for 5 min before staining with Nile Blue as described by Yagihara, Salway & Hawthorne (1969). The ninhydrin-positive spots corresponding to authentic phosphatidylserine and phosphatidylethanolamine were not entirely separated from each other (and were therefore combined for subsequent analysis) but were distinct from phosphatidylcholine and the other phospholipids.

The incorporation of [^{32}P]orthophosphate into the phospholipids of control and local anaesthetic-treated rabbit vagus nerve is summarized in Table 1. Amethocaine, lignocaine, tropacocaine and procaine increased incorporation of ^{32}P into MPI by 185%, 100%, 233% and 59% respectively. Labelling of triphosphoinositides (TPI), diphosphoinositides (DPI) and other phospholipids was not affected in a statistically significant manner.

The increased incorporation of ^{32}P into MPI was not greatly dissimilar both for all the four local anaesthetics used in these experiments (1·6–3·3 fold increase) and for

Table 1. *The incorporation of [^{32}P]orthophosphate (pmol P incorporated mg^{-1} fresh tissue in 62 min: Mean ± standard error) into the phospholipids of rabbit vagus nerve in the presence and absence of various local anaesthetics.* N= number of nerves in each group. Statistically significant differences from the control group (Student's *t*-test) are indicated as follows: * $P < 0{\cdot}05 > 0{\cdot}01$; ** $P < 0{\cdot}01 > 0{\cdot}001$; *** $P < 0{\cdot}001$. The following abbreviations have been used:— Monophosphoinositide (MPI), diphosphoinositide (DPI), triphosphoinositide (TPI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC).

	pmol P incorporated mg^{-1} fresh tissue in 62 min				
Phospholipids	Control (N = 8)	Amethocaine 0·16 mM (N = 6)	Lignocaine 1·42 mM (N = 6)	Tropacocaine 2·39 mM (N = 6)	Procaine 2·82 mM (N = 5)
		***	*	**	*
MPI	1·1 ± 0·20	3·14 ± 0·26	2·21 ± 0·37	3·66 ± 0·73	1·75 ± 0·13
DPI	4·8 ± 0·45	5·79 ± 1·31	6·03 ± 1·12	6·88 ± 1·59	4·04 ± 0·48
TPI	5·84 ± 0·49	6·93 ± 0·71	6·74 ± 0·83	7·42 ± 1·47	5·00 ± 0·45
PS & PE	0·53 ± 0·04	0·65 ± 0·09	0·64 ± 0·04	0·40 ± 0·11	0·35 ± 0·13
PC	1·8 ± 0·20	1·63 ± 0·17	1·64 ± 0·17	1·46 ± 0·20	1·51 ± 0·08

0·063 mM cinchocaine (3·5 fold increase: Salway & Hughes, 1972) although the concentration of the local anaesthetics needed to produce these effects varied over a 45 fold range. Those local anaesthetics with a fast onset of action (e.g. procaine) produced the smallest effect whilst those with a slow onset of action (e.g. cinchocaine) produced the largest effect. Since the concentrations of the local anaesthetics were chosen for their ability to produce complete blockade of axonal conduction within 10 min, it is probable that those acting slowly will have been used at a concentration above their true equilibrium minimum blocking concentration. It is possible that had concentrations of the local anaesthetics been chosen which were equi-effective under equilibrium conditions their effects on MPI labelling might have been even more uniform.

White & Larrabee (1973) have recently shown that δ-hexachlorocyclohexane blocked conduction along nerve trunks whereas γ-hexachlorocyclohexane, although blocking transmission at sympathetic ganglia, had no inhibitory effect on axonal conduction. The pharmacology of δ-hexachlorocyclohexane is not fully understood but one interpretation of the results of White & Larrabee is that δ-hexachlorocyclohexane may prevent axonal conduction by a local anaesthetic type of action. They found the δ-isomer to stimulate incorporation of ^{32}P into MPI in rat superior cervical ganglia by approximately 250% whereas the γ-isomer had no effect on MPI labelling.

These results suggest that the effect on MPI labelling may be related to the potency of the compounds tested as local anaesthetics and support the observations of Eichberg & others (1973). We have no evidence to indicate whether this increase in MPI labelling is a cause or a consequence of blockade of axonal conduction by local anaesthetics but it cannot be a result of blockade of nerve action potentials *per se* since blockade by tetrodotoxin is not accompanied by a change in MPI labelling (Salway & Hughes, 1972).

We are grateful to Dr R. P. Hullin for his support and to Mr. B. V. Booker for technical assistance. J. G. S. is grateful to the Medical Research Council for a grant to Dr R. P. Hullin.

Departments of Pharmacology & Biochemistry,
The Medical School, Thoresby Place
Leeds LS2 9NL, Yorkshire, U.K.

I. E. HUGHES
J. G. SALWAY

May 10, 1973

REFERENCES

EICHBERG, J., SHEIN, H. M. & HAUSER, G. (1973). *Biochem. Soc. Trans.*, **1**, 352–359.
LAPETINA, E. G. & MICHELL, R. H. (1973). *FEBS Lett.*, **31**, 1–10.
SALWAY, J. G. & HUGHES, I. E. (1972). *J. Neurochem.*, **19**, 1233–1240.
WHITE, G. L. & LARRABEE, M. G. (1973). *Ibid.*, **20**, 783–798.
YAGIHARA, Y., SALWAY, J. G. & HAWTHORNE, J. N. (1969). *Ibid.*, **16**, 1133–1139.

Prostaglandins as regulators of bradykinin responses

A number of agents have been studied for their ability to induce the most important changes associated with inflammation—namely, increased permeability of small blood vessels and migration of leucocytes. For example, bradykinin produces the characteristic signs of inflammation in a variety of species and has been identified in inflammatory exudates including those from carrageenan oedema in the rat (as reviewed by Di Rosa & Willoughby, 1971). Both prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) are inducers of local vascular permeability in the rat (Crunkhorn & Willis, 1971; Freeman & West, 1972), and PGE_2 has also been detected in exudates from carrageenan oedema (Willis, 1969).

Ferreira, Moncada & Vane (1973) have recently reported that bradykinin reflexly increases the arterial blood pressure of dogs, proportional to the dose, when injected intra-arterially into the spleen, and that PGE_1 or PGE_2 potentiate these responses when injected together with the bradykinin despite the fact that the prostaglandins by themselves are vasodepressor. Furthermore, indomethacin (a non-steroidal anti-inflammatory drug which inhibits prostaglandin synthesis) reduced the pressor response to bradykinin which was then increased once more by PGE_1 or PGE_2. The authors suggested that the prostaglandins released within the spleen potentiated the nociceptive action of bradykinin.

We have now studied the effects of prostaglandins on the actions of bradykinin, histamine, 5-hydroxytryptamine (5-HT) and clinical dextran (molecular weight 110 000) on blood vessels in the skin of rats. Groups of at least 10 female Wistar rats of the ASH colony (150–210 g) had their backs shaved 24 h before being anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.). After the intravenous injection of azovan blue dye (20 mg kg^{-1}), intradermal injections of PGE_1 PGE_2 or $PGF_{2\alpha}$ alone, or with bradykinin, histamine, 5-HT or dextran, in dose volumes of 0·05 ml Tyrode solution, were made into the shaved areas. Forty-five min later, the rats were killed and the effects of the agents on vascular permeability were measured spectrophotometrically by estimating (in μg) the amount of dye in each weal, using the method of Harada Takeuchi & others (1971). To determine when potentiation or inhibition had occurred, the amounts of dye extracted from the skin after each agent separately were summated and subtracted from the amounts of dye extracted when two agents were given together and the differences were then expressed as percentages of the totals of the agents separately. In each rat, the amount of dye extracted from the skin after control Tyrode injections was subtracted from all other values before calculations