J. Pharm. Pharmacol. 1981, 33: 794–795
Communicated April 14, 1981

0022–3573/81/120794–02 $02.50/0

Effect of basic amine drugs on the metabolism of angiotensin I in rat lung homogenates

P. WORLAND, R. F. MINCHIN, K. F. ILETT*, *Department of Pharmacology, University of Western Australia, Nedlands, 6009 Western Australia*

In mammalian species, the conversion of the decapeptide angiotensin I (AI) to the biologically active octapeptide angiotensin (AII) is catalysed by angiotensin converting enzyme (ACE). ACE has been shown to be located in the luminal membrane of the pulmonary capillary endothelial cells (Ryan et al 1976) and because of its extensive capillary surface area and large blood flow, the lung is the major site for conversion of AI to AII (Soffer 1976). At physiological concentration, the pulmonary clearance of AI is greater than 80% in most species (Gillis & Roth 1976).

Basic amine drugs have been shown to accumulate in lung tissue, particularly during chronic therapy (Brown 1974). Their association with lung is probably due to extensive binding to phospholipids which are present in plasma membranes and vesicles located in the type II alveolar cells (Gillette 1974; Di Francesco & Bickel 1977). The present study was designed to investigate whether basic amine drugs could alter the activity of ACE.

Lungs from male Wistar rats (190–210 g) were homogenized in sucrose (0·25 M) in phosphate (0·02 M) buffer (pH 7·4) and centrifuged at 700 *g* for 10 min. The supernatant was then adjusted to pH 5·2 with 0·1 M acetic acid and centrifuged at 10 000 *g* for 30 min. The resulting pellet was washed once with, and resuspended in, phosphate buffer (0·02 M) containing NaCl (0·17 M) at pH 7·4. AI ($7{\cdot}0 \times 10^{-6}$ M) was incubated for 10 min at 37 °C with lung homogenate (500 μg protein) and inhibitor ($1{\cdot}6 - 2{\cdot}7 \times 10^{-3}$ M) in a final volume of 0·5 ml. The reaction was stopped by the addition of 0·5 ml 0·8 M HCl and AI concentration measured using a commercially available radioimmunoassay procedure (New England Nuclear, U.S.A.) with minor modifications.

Under these conditions, 79·8 ± 11·6% (mean ± s.e.m., n = 14) of the AI was metabolized in 10 min.

Table 1. Effect of various compounds on the metabolism of angiotensin I in rat lung homogenates.

Inhibitor	Concentration mM	% Inhibition mean ± s.e.m. (n)
Chlorimipramine	2·0	87·0 ± 7·1 (7)
Chlorpromazine	2·0	50·9 ± 7·4 (10)
Haloperidol	2·0	45·9 ± 6·8 (10)
Desmethylimipramine	2·0	27·9 ± 2·6 (10)
Propranolol	2·0	9·8 ± 3·7 (10)
Chlorphentermine	2·0	3·0 ± 0·8 (10)
Captopril	2·0	62·0 ± 7·0 (10)
Ethylenediaminetetra-acetic acid	1·6	47·3 ± 3·8 (10)
Dimercaptopropanol	2·7	87·3 ± 3·2 (10)

The results of studies using various known inhibitors of ACE and several basic amine drugs are shown in Table 1. The conversion of AI to AII my be due to both specific endothelial cell membrane-associated ACE and non-specific intracellular peptidases (Bakhle 1974). AI was incubated in the presence of a high concentration of captopril (2×10^{-3} M), a specific ACE inhibitor with little antagonism for other peptidases (Cushman et al 1979). Captopril decreased AI metabolism by 62% suggesting that the remaining 38% at least was due to enzyme systems other than ACE. Two other known inhibitors of ACE were also studied. At a concentration some 16 times that required for a > 90% inhibition of purified ACE (Das & Soffer 1975), ethylenediaminetetra-acetic acid showed a 47% inhibition of AI conversion. Again this suggests that other enzyme systems in our preparation may be contributing to the removal of AI. By contrast, another chelating agent, dimercaptopropanol, was considerably more potent with an inhibition of 87%. Of the basic amine drugs tested chlorimipramine demonstrated the greatest degree of inhibition (87%), chlorpromazine and haloperidol were much less potent (51 and 46% respectively) while propranolol and chlorphentermine had little or no effect.

To our knowledge there are only two reports in the literature of the effects of amines on the conversion of AI to AII. These are paraquat (Roth et al 1979) and nicotine (Toivonen 1980), both of which are capable of causing structural damage to the lung. With paraquat there was a decrease in rat lung ACE activity 4 days after treatment at which time tissue damage due to paraquat can be expected to be significant (Witschii & Cote 1977). By contrast, nicotine failed to alter the production of AII from AI by isolated perfused rat lungs even after 10 days of treatment. The present study has reported preliminary results which indicate that a number of basic amine drugs which are known to be accumulated in lung tissue can decrease the activity of ACE in tissue homogenates. Because such drugs can accumulate to unusually high concentrations in lung during chronic therapy, this finding indicates that they may interfere significantly with the biological role of AII in the body.

The authors wish to thank Wellcome Australia Ltd for financial support.

REFERENCES

Bakhle, Y. S. (1974) in: Page, I. M., Bumpus, F. M. (eds) Angiotensin. Handbook of Experimental Pharmacology, Vol. 37. Springer-Verlag, Berlin, pp 41–80

* Correspondence.

Brown, E. A. B. (1974) Drug Metab. Rev. 3: 33–87

Cushman, D. W., Cheung, M. S., Sabo, E. F., Rubin, B., Ondetti, M. A. (1979) Fed. Proc. Fed. Am. Soc. Exp. Biol. 38: 2778–2782

Das, M., Soffer, R. L. (1975) J. Biol. Chem. 250: 6762–6768

Di Francesco, C., Bickel, M. H. (1977) Chem.-Biol. Interactions 16: 335–346

Gillette, J. R. (1974) in: Teorell, T., Dedrick, R. L., Condliffe, P. G. (eds) Pharmacology and Pharmacokinetics. Plenum Press, New York, pp 209–233

Gillis, C. N., Roth, J. A. (1976) Biochem. Pharmacol. 25: 2547–2553

Roth, R. A., Wallace, K. B., Alper, R. M., Bailie, M. D. (1979) Ibid. 28: 2349–2355

Ryan, U. S., Ryan, J. W., Whitaker, C., Chiu, A. (1976) Tissue Cell 8: 125–145

Soffer, R. L. (1976) Biochem. Rev. 45: 73–94

Toivonen, M. (1980) Res. Commun. Chem. Pathol. Pharmacol. 28: 563–566

Witschii, H., Cote, M. G. (1977) CRC Crit. Rev. Toxicol. 5: 23–66

J. Pharm. Pharmacol. 1981, 33: 795–796
Communicated May 6, 1981

0022–3573/81/120795–02 $02.50/0

Effect of oximes and atropine on the concentration of cerebral glycogen and blood glucose in malathion-treated rats

RITA AGARWAL, M. A. MATIN*, *Industrial Toxicology Research Centre, Post Box 80, Lucknow, U.P., India.*

Organophosphorus compounds inhibit cholinesterase and produce toxic effects such as hyperexcitability, tremors and convulsions (Stewart 1952; Nachmansohn 1959); these effects are controlled by oximes which have been reported to reactivate the phosphorylated (inhibited) cholinesterase (Wilson & Ginsburg 1955; Child et al 1955). Although certain organophosphorus compounds have been reported to produce hyperglycaemia (Dybing & Sognen 1958; Holmstedt 1959; Weiss et al 1964), the effect of oximes on this state has not been determined. We have examined the effect of atropine and two oximes, 2-formyl 1-methyl pyridinium oxime chloride (2-PAM) and diacetyl monoxime (DAM) on the concentration of blood glucose in malathion-treated rats; cerebral glycogen concentration in some of the animals was also determined.

Methods

Adult female albino rats, 150 ± 10 g, fasted for 18 h before use (since this was found to give more uniform results) were divided into several groups. The animals of group I were treated with malathion (500 mg kg^{-1} i.p). The animals of group II received malathion followed immediately or after 15 min by 2-PAM or DAM (each, 100 mg kg^{-1} i.p). The animals of the group III were given malathion followed immediately by atropine (75 mg kg^{-1} i.p). The animals of group IV were treated with reserpine (1·0 mg kg^{-1} i.p.) daily for three days before the administration of malathion. Controls had 0·9% NaCl. The animals were decapitated 1 h after treatment with malathion. Blood glucose was determined by the method of Nelson (1944). Cerebral glycogen was extracted according to Lebaron (1955) and estimated colorimetrically (Montgomery 1957). The data were analysed using Student's *t*-test.

* Cor spondence.

Results and discussion

The level of blood glucose was raised and cerebral glycogen reduced after treatment with malathion (Table 1). 2-PAM or DAM given immediately after malathion prevented the increase in blood glucose but when given 15 min later, the

Table 1. Effect of oximes (2-PAM, DAM) and atropine on the level of cerebral glycogen and blood glucose in malathion-treated rats. Each group consisted of eight animals. All the animals were killed 1 h after treatment with malathion.

	Malathion 500 mg kg^{-1} i.p., followed by							
	(1) Controls	(2) None	(3) 2-PAM 100 mg kg^{-1} (after 15 min)	(4) 2-PAM 100 mg kg^{-1} (immediately)	(5) DAM 100 mg kg^{-1} (after 15 min)	(6) DAM 100 mg kg^{-1} (immediately)	(7) Atropine 75 mg kg^{-1} (immediately)	(8) Reserpine*
Blood glucose (mg/100 ml) mean ± s.e.	97·33 ± 3·46	212·83[a] ± 11·36	188·93[a] ± 6·59	111·16[b,c] ± 3·24	197·88[a] ± 6·33	106·37[b,c] ±3·90	111·63[b,c,d] ±1·41	209·75[a] ± 13·75
Glycogen (mg/100 g) mean ± s.e.	100·53 ± 3·51	74·83[a] ± 3·81	80·39[a] ± 4·26	99·61[b,c] ± 4·05	75·61[a] ± 4·53	104·06[b,c,d] ± 5·05	99·94[b,c] ± 3·37	78·08[a] ± 3·79

* Reserpine (1 mg kg^{-1} i.p.) was given daily for 3 days before the administration of malathion.
[a] Significantly different from the control values (group 1), $P < 0·01$.
[b] Significantly different from the values in malathion treated animals (group 2), $P < 0·01$.
[c] Significantly different from the values in group 3, $P < 0·01$.
[d] Significantly different from the values in group 5, $P < 0·05$.