Brit. J. Pharmacol. (1961), **17**, 261-266.

SOME EFFECTS OF A MONOAMINE OXIDASE INHIBITOR UPON CHANGES PRODUCED BY CENTRALLY ADMINISTERED AMINES

BY

R. J. SCHAIN*

From the National Institute for Medical Research, Mill Hill, London, N.W.7

(*Received July* 19, 1961)

In cats treated with subcutaneous or intraperitoneal injections of the monoamine oxidase inhibitor phenylisopropylhydrazine, the effects of 5-hydroxytryptamine injected into the lateral cerebral ventricle were greatly intensified and prolonged; the effects of adrenaline were potentiated to a lesser extent. Those of noradrenaline, dopamine and tryptamine were not intensified and were prolonged to only a slight degree.

One of the problems involved in understanding the role of naturally occurring amines in the brain is concerned with their physiological inactivation. The enzyme monoamine oxidase has been thought to play the major role in this inactivation, but its physiological significance has become progressively more difficult to assess as evidence accumulates that alternative mechanisms exist for the metabolism of biogenic amines (Sjoerdsma, 1959). In addition, the substrate specificity of monoamine oxidase varies considerably according to the source of the enzyme (Hagen & Weiner, 1959). Drugs characterized as monoamine inhibitors are useful in counteracting certain depressive states, and this has helped to maintain strong interest in the role of this enzyme in central nervous system function. Doubt, however, has been expressed concerning the view that the clinical effects of these inhibitors result from their action on this enzyme (Rosenblum & Ferguson, 1960), as many other properties of the hydrazine monoamine oxidase inhibitors are known (Horita, 1961).

The behavioural effects of amines injected into the cerebral ventricles have been described by several investigators (Feldberg & Sherwood, 1954; Gaddum & Vogt, 1956; Bradley, 1958). A major advantage of the intraventricular method of administration is that it allows the direct observation of central effects of drugs without the complication of peripheral vascular effects.

In the present experiments, the effect of pretreatment with a hydrazine monoamine oxidase inhibitor (phenylisopropylhydrazine) was examined upon behavioural and other changes occurring after the intraventricular administration of catecholamines

* Special Fellow of the National Institute of Neurological Diseases and Blindness, U.S.A. Present address: Section of Neurology, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

and indoleamines. In some experiments, the effect of 5-hydroxytryptamine was examined after pretreatment with reserpine.

METHODS

The amines were injected into the lateral cerebral ventricle of conscious cats through an indwelling Collison cannula. The method of implantation of the cannula under anaesthesia and aseptic conditions was that described by Feldberg & Sherwood (1953).

The amines were given in a volume of 0.25 ml. of saline solution. An interval of at least one week was allowed between each injection. The amines used were adrenaline and noradrenaline bitartrate, 3-hydroxytyramine (dopamine) hydrochloride, 5-hydroxytryptamine creatinine sulphate, and tryptamine hydrochloride. All doses refer to the free bases.

Phenylisopropylhydrazine (kindly supplied by Lakeside Laboratories, Milwaukee, U.S.A.) was given subcutaneously or intraperitoneally in daily doses of 2 mg/kg for up to three days. The amines were tested by the intraventricular route 2 to 4 or 24 hr after the last injection. Reserpine (Serpasil, Ciba) was given subcutaneously as a single injection; two cats received 0.05 mg/kg and two 0.2 mg/kg. The amines were tested by the intraventricular route 2 hr later.

RESULTS

Intraventricular injection of catecholamines and indoleamines. The intraventricular administration of 40 to 200 μg of adrenaline or noradrenaline resulted in drowsiness and disappearance of spontaneous activity. The cats would crouch in their cages, head nodding forward and eyes tending to close. Tachypnoea was regularly seen. Occasionally coarse or fine tremor was present in head and trunk. When pushed gently with a small rod, the cats became immediately wakeful. When they attempted to walk, ataxia was evident. Doses of over 100 μg often produced vomiting or retching. As shown in Table 1, the duration of the effects was slightly longer with adrenaline than with noradrenaline.

The effects of dopamine were similar to those of adrenaline and noradrenaline, but less intense. With doses of 200 μg the effects usually lasted 30 min to 2 hr (Table 1).

Intraventricular 5-hydroxytryptamine produced tachypnoea, loss of spontaneous activity, ataxia and often strong tremor. Drowsiness or vomiting was not observed. The duration of the effects of various doses (10 to 200 μg) is given in Table 1.

Tryptamine produced effects similar to, but less intense than, those produced by 5-hydroxytryptamine. Tremor was not seen. The effect of 200 μg lasted 30 min to 2 hr (Table 1).

Intraventricular injection of catecholamines and indoleamines after phenylisopropylhydrazine. Daily subcutaneous or intraperitoneal administration of 2 mg/kg of phenylisopropylhydrazine produced ataxia. This effect has been described by Spector, Shore & Brodie (1959) and by Vogt (1960). Ataxia appeared on the third day, but was not severe enough to prevent the cats from walking freely. Some animals developed profuse salivation, and appeared to be apprehensive and hyperexcitable. All effects disappeared within 24 hr after the last injection of phenylisopropylhydrazine.

TABLE 1

DURATION OF EFFECTS OF INTRAVENTRICULAR AMINES BEFORE AND 2 TO 4 HR, OR 24 HR,* AFTER THE LAST DAILY INJECTION OF 2 MG/KG PHENYLISOPROPYL-HYDRAZINE (PIH)

Compound and amount in μg given intraventricularly		Administration of PIH		Duration of effect in hr		
		Days	Route	Before	After PIH	
Adrenaline	40	1	Intraperitoneally	1	1	
	100	1		3	20	
	100	1		2	6	
	150	3	Subcutaneously	2	40	(death)
	200	3	Intraperitoneally	2	4	
	200*	3	Subcutaneously	3	24	
	200*	3		10	8	
	200*	3		4	10	
Noradrenaline	50	3		½	½	
	50	3		0	2	
	100	3		½	4	
	125	3		1	4	
	200*	3		3	6	
	200*	3		2	½	
	200*	3		½	4	
Dopamine	200	1	Intraperitoneally	0	3	
	200	1		2	6	
	200	1		½	1	
	200	1		2	4	
	200*	3	Subcutaneously	3	5	
	200*	3		1	1	
	200*	3		½	2	
5-Hydroxytryptamine	10	3		½	1	
	10	3		½	2	
	25	3		1	24	
	50	3		½	4	
	50	3		1	192	
	100	2		2	4	
	125	1	Intraperitoneally	0	1	
	200	1		2	72	(seizures)
	200	1		2	48	
	200	1		4	15	
	200	1		6	4	
	200*	3	Subcutaneously	4	48	(death)
	200*	3		2	24	
Tryptamine	200	3	Intraperitoneally	½	3	
	200	3		2	2	
	200	3		1	3	
	200	3	Subcutaneously	½	2	
	200	3		0	0	

After the treatment with phenylisopropylhydrazine the effects of intraventricular noradrenaline, dopamine and tryptamine lasted slightly longer (see Table 1). The sedative effect of noradrenaline appeared to be less pronounced, otherwise the effects of these amines were unchanged.

Treatment with the inhibitor intensified the ataxia and muscular weakness, but appeared to lessen the sedative effect of intraventricular adrenaline. The incidence of vomiting and retching was unchanged. The duration of the effects was prolonged to varying degrees. In three of the eight experiments shown in Table 1 the effects lasted for 20 to 40 hr. In these animals ataxia was severe and there was evidence of hind-limb weakness. The cats lay on their side or abdomen, and were unable

to crouch, sit or walk. One animal died. Severe effects of this kind have been described by Rothballer (1959) following intraventricular adrenaline alone when injected in a much larger dose (2 mg).

The treatment with phenylisopropylhydrazine intensified and prolonged the effects of intraventricular 5-hydroxytryptamine to a much greater extent than those of any of the other amines examined. Before the phenylisopropylhydrazine treatment the effects never lasted for more than a few hours; afterwards they persisted in half the experiments for 1 to 8 days (Table 1). During this time, the animals lay in their cages, unable to sit or stand or even crawl about. There was generalized muscular flaccidity although the patellar tendon reflex was unchanged. The animals were awake; their eyes were open and followed the observer. The only responses to gentle prodding were slight turning of the head and weak cries. The animals ate poorly and had to be fed with diluted milk. All except one animal recovered. Another animal developed recurrent seizures which began with twitching of the facial muscles and developed into brief generalized tonic-clonic convulsions. These ceased spontaneously after the postural effects had disappeared. There were short periods of twitching of the facial muscles in two other cats.

Intraventricular injection of 5-hydroxytryptamine after reserpine. The subcutaneous injection of 0.05 or 0.2 mg/kg of reserpine resulted in miosis and sedation as first described by Bein (1953). Miosis developed within 30 min and was followed by crouching and closing of the eyes. The animals were sluggish but could be induced to walk by gentle prodding. Fine tremors were noted at times. Signs of recovery were evident by 6 hr and full recovery occurred within 24 to 48 hr.

The reserpine treatment increased the tremor produced by intraventricular injection of 200 μg of 5-hydroxytryptamine. Two of the treated cats uttered intermittent cries during the first hour after the 5-hydroxytryptamine injection, but its other responses could not be distinguished from the general effects of subcutaneous reserpine; there was no evidence that they were changed or intensified.

DISCUSSION

The most striking observation was the intensification and prolongation of the effects of intraventricular 5-hydroxytryptamine seen in some of the cats pretreated with the monoamine oxidase inhibitor phenylisopropylhydrazine. This potentiation and prolongation cannot be explained by summation of the effects of the amines with those of phenylisopropylhydrazine and is likely to be the outcome of inhibition of monoamine oxidase activity. Potentiation of the responses to adrenaline also occurred after phenylisopropylhydrazine, but to a lesser extent. Intensification of the effects of noradrenaline, dopamine or tryptamine after phenylisopropylhydrazine did not occur, and their prolongation was so slight that it is not possible to decide whether this was due to a longer-lasting action of these amines or to summation of the effects with those of phenylisopropylhydrazine.

It is well known that tryptamine is less potent than 5-hydroxytryptamine on many smooth muscle preparations. This difference in potency also applies to the central effects observed after the intraventricular injection of these amines. A possible

explanation for the difference in potency of these two amines on peripheral structures has been proposed by Vane (1959). He has suggested that, since tryptamine is more fat-soluble than 5-hydroxytryptamine, it is likely to pass cellular diffusion barriers more readily than 5-hydroxytryptamine, and therefore would be more rapidly inactivated by intracellular amine oxidase. The present results do not support this explanation as applied to the central effects of these amines administered into the cerebral ventricles. If contact with amine oxidase were the decisive factor, the effects of tryptamine should have been potentiated to a greater extent after phenylisopropylhydrazine than those of 5-hydroxytryptamine. Since this did not occur in these experiments, inaccessibility to the action of monoamine oxidase is here not a consequence of the presence of the phenolic hydroxyl group in 5-hydroxytryptamine.

According to Goldberg (1959), the cardiovascular actions of dopamine and tryptamine are more significantly potentiated by monoamine oxidase inhibitors than those of adrenaline, noradrenaline or 5-hydroxytryptamine. In the present experiments the order in which phenylisopropylhydrazine potentiated the central effects of these amines was different: the effects of dopamine and tryptamine were hardly affected whereas those of adrenaline and 5-hydroxytryptamine were significantly increased. This apparent difference in potentiation of these amines suggests that the influence of enzyme inhibition on the effects of pharmacologically active substrates in the central nervous system differs from that in peripheral tissues.

Two alternatives may account for the fact that there was considerable individual variation in the effects of the intraventricular injection of the amines after pretreatment with phenylisopropylhydrazine. One is that inhibition of monoamine oxidase *in vivo* is incomplete and that the effects are dependent upon the degree of inhibition in each animal. The other is that the amines can be disposed of by means other than amine oxidase activity, such as simple diffusion away from their sites of action in the brain, their uptake by neighbouring tissues, or their metabolism via other pathways as exemplified by O-methylation of the catecholamines (Axelrod, 1959).

The author is indebted to Sir Charles Harington for hospitality, and to Professor W. Feldberg for his interest during these experiments.

REFERENCES

AXELROD, J. (1959). Metabolism of epinephrine and other sympathomimetic amines. *Physiol. Rev.*, **39**, 751–776.

BEIN, H. J. (1953). Zur Pharmakologie des Reserpin, eines neuen Alkaloids aus Rauwolfia Serpentina Benth. *Experientia*, **9**, 107–110.

BRADLEY, P. B. (1958). The effects of 5-hydroxytryptamine on the electrical activity and on behaviour in the conscious cat. In *5-Hydroxytryptamine*, pp. 214–220, ed. G. P. LEWIS. London : Pergamon.

FELDBERG, W. & SHERWOOD, S. L. (1953). A permanent cannula for intraventricular injections in cats. *J. Physiol. (Lond.)*, **102**, 3–4*P*.

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

GADDUM, J. H. & VOGT, M. (1956). Some central actions of 5-hydroxytryptamine and various antagonists. *Brit. J. Pharmacol.*, **11**, 175–179.

GOLDBERG, L. I. (1959). Pharmacology of iproniazid and other amine oxidase inhibitors. *Ann. N.Y. Acad. Sci.*, **80**, 639–642.

HAGEN, P. & WEINER, N. (1959). Enzymic oxidation of pharmacologically active amines. *Fed. Proc.*, **18**, 1005–1012.

HORITA, A. (1961). Some pharmacological aspects of monoamine oxidase regulators. *J. Neuropsychiat.*, **2**, *S*141–145.

ROSENBLUM, I. & FERGUSON, F. C. Jr. (1960). Monoamine oxidase inhibition. *Clin. Pharmacol. & Therap.*, **1**, 692–693.

ROTHBALLER, A. B. (1959). The effects of catecholamines on the central nervous system. *Pharmacol. Rev.*, **11**, 494–547.

SJOERDSMA, A., LOVENBERG, W., OATES, J. A., CROUT, J. R. & UDENFRIEND, S. (1959). Alterations in the pattern of amine excretion in man produced by a monoamine oxidase inhibitor. *Science*, **130**, 225.

SPECTOR, S., SHORE, P. A. & BRODIE, B. B. (1959). Biochemical and pharmacological effects of the monoamine oxidase inhibitors, iproniazid, 1-phenyl-2-hydrazinopropane (JB–516) and 1-phenyl-3-hydrazinobutane (JB–835). *J. Pharmacol. exp. Ther.*, **127**, 15–21.

VANE, J. R. (1959). The relative activities of some tryptamine analogues on the isolated rat stomach strip preparation. *Brit. J. Pharmacol.*, **14**, 87–98.

VOGT, M. (1960). In *Adrenergic Mechanisms*, p. 575. Ciba Foundation Symposium, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, MAEVE. London : J. & A. Churchill Ltd.