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Effect of monoamineoxidase inhibitors on 5-hydroxytryptamine output from perfused cerebral ventricles of anaesthetized cats

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1. In cats anaesthetized with pentobarbitone sodium, intraperitoneal injections of four inhibitors of monoamine oxidase (MAO) were shown to increase the 5-hydroxytryptamine (5-HT) in the effluent from the perfused cerebral ventricles.

2. Weight for weight, tranylcypromine was found to be about twice as potent as pheniprazine, eight times as potent as nialamide and sixty times as potent as pargyline.

3. The effect of tranylcypromine was also examined after reserpine had been injected into the cerebral ventricles or after *p*-chlorophenylalanine, given intraperitoneally. In both conditions tranylcypromine retained its ability to increase the 5-HT output from the perfused cerebral ventricle, but the effect was attenuated, more after *p*-chlorophenylalanine than after reserpine.

4. Evidence is put forward that in both conditions the brain is not completely depleted of its 5-HT, but that the 5-HT is only reduced, more after *p*-chlorophenylalanine than after reserpine.

Recently it was shown that an intraperitoneal injection of tranylcypromine, an inhibitor of monoamine oxidase (MAO) increased the 5-hydroxytryptamine (5-HT) content in the effluent from the perfused cerebral ventricles of anaesthetized cats (El Hawary, Feldberg & Lotti, 1967). The increase was attributed to inhibition of the MAO. As the 5-HT released in the ventricular walls is no longer destroyed after inhibition of the enzyme it would diffuse in greater amounts into the fluid perfusing the ventricles. However, tranylcypromine has strong stimulating amphetamine-like actions which might increase the release of 5-HT. This, too, would result in an increased output in the effluent. This possibility would be excluded as the cause for the observed increase, if MAO inhibitors without amphetamine-like actions also were to increase the 5-HT output, and if their ability to do so were related to their potency as inhibitors of MAO.

In the present experiments, four inhibitors of different potency were used. Two of them, tranylcypromine and pheniprazine, have strong amphetamine-like actions,

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whereas the other two, nialamide and pargyline, are without these actions (Goldberg & Sjoerdsma, 1959; Kinross-Wright, 1959; Spencer, 1960; Lee, Shin & Shideman, 1961; Goldberg, 1964). Their effects were compared on the 5-HT output from the perfused cerebral ventricles of anaesthetized cats, and the effect of tranylcyromine was also studied in cats in which the 5-HT level of the brain had been reduced either by reserpine injected into the cerebral ventricles or by *p*-chlorophenylalanine injected intraperitoneally.

Methods

In cats of both sexes weighing between 2·3 and 3·5 kg, the cerebral ventricles were perfused with artificial cerebiospinal fluid from the left cannulated lateral ventricle to the cannulated aqueduct according to the method described by Carmichael, Feldberg & Fleischhauer (1964). Cannulation of the lateral ventricle was performed aseptically under pentobarbitone sodium anaesthesia at least 3 days before the perfusion as in the experiments of Banerjee, Feldberg & Lotti (1968) to avoid temperature effects produced by inserting the cannula. For the perfusion, the cats were again anaesthetized with an intraperitoneal injection of pentobarbitone sodium (33 mg/kg). Perfusion was begun at a rate of 0·1 ml./min, but reduced to 0·05 ml./min as soon as a satisfactory outflow from the aqueductal cannula was established. The perfusion fluid was the artificial cerebrospinal fluid of Merlis (1940). During the perfusion the pressure was measured from a side arm of the inflow tubing with a strain gauge transducer. In the experiments to be described, the pressure did not rise more than 10 cm H_2O during the perfusion. The effluent was collected as half-hour samples and the collecting tubes were cooled on ice during collection and kept on ice until the samples were assayed for 5-HT on the rat fundus strip according to the method of Vane (1957).

The MAO inhibitors used were tranylcypromine sulphate, pheniprazine (β-phenylisopropylhydrazine hydrochloride), nialamide and pargyline hydrochloride. They were injected intraperitoneally in a volume of 2 ml. The doses refer to the salt. Reserpine phosphate, kindly supplied by Dr. A. J. Plummer, Ciba Pharmaceutical Company, Summit, New York, U.S.A., was dissolved in pyrogen-free distilled water, the solution was kept in the dark and cold but was warmed to room temperature before being injected into the cannulated lateral ventricle in a dose of 0·5 mg dissolved in 0·1 ml. followed by an injection of 0·05 ml. 0·9% NaCl solution to wash in the drug. *p*-Chlorophenylalanine, kindly supplied by Dr. H. Reinert, Pfizer Ltd., Sandwich, Kent, was injected intraperitoneally in a dose of 300 mg/kg suspended in 2 ml. pyrogen-free 0·9% NaCl solution.

Results

On perfusion of the cerebral ventricles at an infusion rate of 0·05 ml./min the effluent collected from the cannulated aqueduct during each half-hour amounted usually to 1·8 ml. Sometimes the first half-hour sample was tinged with blood and gave higher 5-HT values when tested on the fundus strip of the rat stomach than the following sample. Column 1 of Table 1 shows that in the twenty-two experiments in which the 5-HT of the second half-hour sample was assayed it amounted to between 0·3 and 1·2 ng/ml. (mean 0·6 ng/ml.). When no MAO inhibitor was given, the 5-HT content remained practically unchanged in the subsequent samples for at least 4 hr.

5-HT output after monoamine inhibitors

Tranylcypromine. The finding of El Hawary *et al.* (1967) was confirmed that an intraperitoneal injection of tranylcypromine increases the 5-HT content in the effluent from the perfused cerebral ventricles. The results obtained with 2·5 and

TABLE 1. *Effect of intraperitoneal injections of MAO inhibitors on 5-HT output in the effluent from the perfused cerebral ventricles of anaesthetized cats.*

Control						
	Tranylcypromine 2·5 mg/kg					
0·6	0·7	1·6	2·0	2·5	4·3	4·2
0·8	1·2	1·5	3·6	3·2	3·4	3·4
0·6	0·5	1·5	2·2	4·1	4·8	4·5
0·4	0·5	1·5	2·9	5·5	6·6	7·5
Mean 0·6	0·8	1·5	2·7	3·8	4·8	4·9
	Tranylcypromine 5 mg/kg					
0·9	1·6	5·8	7·5	8·7	10·4	8·3
0·8	0·8	1·8	2·9	5·2	6·3	7·5
0·7	0·7	1·4	2·3	2·2	2·9	3·3
0·4	1·0	2·5	3·3	2·6	2·9	
1·0	2·0	5·2	6·3	6·3		
0·6	2·5	2·5	4·0	3·3	5·0	
Mean 0·8	1·5	3·2	4·4	4·7	5·5	6·4
	Pheniprazine 5 mg/kg					
0·9	1·0	2·0	3·3	3·3	3·3	3·3
0·3	1·0	1·2	2·5	2·0	2·5	2·5
0·5	1·0	3·8	4·6	5·6	5·6	
Mean 0·6	1·0	2·6	3·5	3·6	3·8	2·9
	Nialamide 2·5 mg/kg					
1·2	1·0	1·8	1·8	2·0	1·5	1·2
0·8	0·8	0·8	0·8	0·8	0·8	0·8
Mean 1·0	0·9	1·3	1·3	1·4	1·7	1·0
	Nialamide 10 mg/kg					
0·9	1·0	1·3	1·3	2·0	2·2	2·9
0·6	0·6	0·6	0·8	1·0	1·3	1·2
0·5	1·0	1·0	1·0	2·0	1·9	2·2
0·8	1·6	1·0	1·2	1·2	1·4	1·4
Mean 0·7	1·0	1·0	1·1	1·6	1·7	1·8
	Pargyline 75 mg/kg					
	1·3	1·7	2·0	2·0	2·0	2·5
0·3		0·6		0·8	1·2	1·3
Mean 0·3	1·3	1·2	2·0	1·4	1·6	1·9
	Pargyline 150 mg/kg					
0·5	0·5	2·0	2·0	3·5	3·5	3·5
0·6	1·0	2·0	3·5	5·0	6·0	6·0
Mean 0·6	0·8	2·0	2·8	4·3	4·8	4·8

The horizontal line of figures represents 5-HT content in ng/ml. of successive half-hour samples for each animal. Column 1 (control) the 5-HT equivalent in the last of two samples collected before, columns 2–7 in the six successive samples collected after the tranylcypromine injection.

5 mg/kg are shown in the first ten experiments of Table 1. With the smaller dose the increase occurred a little later than with the larger dose, that is, usually not until the second instead of in the first half-hour sample collected after the intraperitoneal injection. As seen from a comparison of the mean values, the effect of 5 mg/kg was stronger than that of 2·5 mg/kg, but in individual experiments the increase produced by 2·5 mg/kg was as great or greater than that produced in some experiments by 5 mg/kg.

Pheniprazine, nialamide and pargyline. These three MAO inhibitors also increased the 5-HT output from the perfused cerebral ventricles, but they were less potent than tranylcypromine (see Table 1). From the mean values, it is evident that pheniprazine 5 mg/kg caused about the same rise in 5-HT output as tranylcypromine 2·5 mg/kg. Nialamide was even less potent. In one of two experiments in which 2·5 mg/kg was injected intraperitoneally, the 5-HT output did not increase, in the other the increase was small, and after nialamide 10 mg/kg the increase was not as great as after tranylcypromine 2·5 mg/kg. The least potent inhibitor was pargyline which had to be injected in much larger doses in order to raise the 5-HT output. The last four experiments of Table 1 show the effects of intraperitoneal injections of pargyline 75 and 150 mg/kg. The rise produced by 75 mg/kg was about the same as that obtained with nialamide 10 mg/kg, and the rise produced by 150 mg/kg was about the same as that obtained with tranylcypromine 2·5 mg/kg. Thus weight for weight, pargyline was about 1/60, nialamide 1/8 and pheniprazine about 1/2 as potent as tranylcypromine.

TABLE 2. *Effect of intraperitoneal injections of tranylcypromine on 5-HT output in the effluent from the perfused cerebral ventricles of anaesthetized cats pre-treated with two intraventricular injections of* 0·5 *mg reserpine (first eight experiments) or with one intraperitoneal injection of* 300 *mg/kg* p-*chlorophenylalanine (last five experiments).*

Control	Tranylcypromine 2·5 mg/kg after reserpine					
0·4	0·4	0·6	1·0			
0·6	0·7	1·0	1·3	1·5	2·1	2·1
0·6	0·4	0·6	0·9	1·5	2·3	3·3
0·5	0·6	1·0	1·2	1·5	1·3	1·5
Mean 0·5	0·5	0·8	1·1	1·5	1·9	2·3
	Tranylcypromine 5 mg/kg after reserpine					
0·3	0·4	1·0	1·0	1·0	1·0	1·0
0·4	1·3	2·8	3·5	3·1	3·1	3·1
0·8	0·9	1·0	2·0	2·5	3·1	3·6
0·4	1·0	1·6	3·3	4·0	3·6	3·3
Mean 0·5	0·9	1·6	2·5	2·7	2·7	2·8
	Tranylcypromine 2·5 mg/kg after *p*-chlorophenylalanine					
0·25	0·5	0·6	0·6	0·5		1·0
0·6	0·6	1·0	1·3	2·0	1·7	1·7
	Tranylcypromine 5 mg/kg after *p*-chlorophenylalanine					
0·3	0·4		0·8	0·8	1·1	1·3
0·25	0·4	0·5	1·0	1·0	1·0	1·0
0·1	0·6	2·5	2·5	2·5		

The horizontal lines of figures represent 5-HT content in ng/ml. of successive half hour samples for each animal. Column 1 (Control) the 5-HT equivalent in the last of two samples collected before, columns 2–7 in the six successive samples collected after the tranylcypromine injection.

5-HT output after treatment with monoamine depleting substances

Reserpine. When the cerebral ventricles were perfused 18 hr after the last of two intraventricular injections of 0·5 mg reserpine, given at a 24 hr interval, the 5-HT content of the effluent was about the same as in cats not treated with reserpine. The 5-HT equivalent varied between 0·3 and 0·8 ng/ml. (mean 0·5 ng/ml.) in the second half-hour sample of the first eight experiments of Table 2. Tranylcypromine retained its ability to increase the 5-HT output from the perfused ventricles, but its effect was not as great as in cats not treated with reserpine. In the reserpinized cats the mean 5-HT output rose to 2·3 ng/ml. after the injection of 2·5 mg/kg and to 2·8 ng/ml. after the injection of tranylcypromine 5 mg/kg as compared with 4·9 ng/ml. and 6·4 ng/ml. in the untreated cats.

Another difference was found when perfusion was continued after death. El Hawary *et al.* (1967) had found that when cats were killed a few hours after an intraperitoneal injection of tranylcypromine, there was a large increase in the already high 5-HT content of the samples collected after death. In three cats pre-treated with intraventricular reserpine this did not happen when they were killed a few hours after an intraperitoneal injection of tranylcypromine 5 mg/kg, and perfusion of the cerebral ventricles was continued for 2 hr after death. In two cats the 5-HT did not increase and in the third the increase was small.

p-*Chlorophenylalanine.* When the cerebral ventricles were perfused 3 days after an intraperitoneal injection of *p*-chlorophenylalanine 300 mg/kg, the effluent contained less 5-HT than in experiments on cats that had not received *p*-chlorophenylalanine, and even less than in cats treated with reserpine. The results of five experiments are shown in Table 2. In two of them no 5-HT was detected in the second half-hour samples; each certainly contained less than 0·25 ng/ml. which would have been detected with the sensitivity of the fundus strip preparations used for the 5-HT assay in these two experiments. In the other three experiments the 5-HT equivalent of the second half-hour sample was 0·6, 0·3 and 0·1 ng/ml. In all five experiments an intraperitoneal injection of tranylcypromine 2·5 or 5 mg/kg increased the 5-HT output but the rise was smaller than in cats not treated with *p*-chlorophenylalanine, and even smaller than in cats treated with reserpine.

Discussion

The present experiments were undertaken to find out whether the increase in 5-HT output from the perfused cerebral ventricles of anaesthetized cats following an intraperitoneal injection of tranylcypromine resulted from its MAO-inhibiting or from its amphetamine-like reaction. The finding that MAO inhibitors increased the 5-HT output independently of their amphetamine-like action, suggests that inhibition of the brain MAO is the sole mechanism by which this effect is produced. In that case, the ability of the four inhibitors used in the present experiments to increase the 5-HT output should parallel their MAO inhibiting property. Weight for weight, tranylcypromine was found to be about twice as potent as pheniprazine, about eight times as potent as nialamide, and about sixty times as potent as pargyline, in increasing the 5-HT output on intraperitoneal injection. Since the degree of MAO inhibition varies in different tissues—for instance, the inhibition of brain MAO does not always follow that of the liver (Horita, 1961)—and may vary from species to species, the comparison should be based on *in vitro* determinations of the

action of the inhibitors on the MAO of the cat's brain. Such determinations are not available but some idea of the potency is derived from experiments in which the increase in the 5-HT concentration in the cat's brain was examined after their daily subcutaneous or intraperitoneal injection. A rise in the 5-HT concentration of the cat's brain was obtained by Funderburk, Finger, Drakontides & Schneider (1962) with daily subcutaneous injections of tranylcypromine 2–5 mg/kg and of nialamide 10 mg/kg, and by Brodie, Spector & Shore (1959) with daily subcutaneous injections of pheniprazine 2 mg/kg and by Schoepke & Wiegand (1963) with daily intraperitoneal injections of pargyline 75 mg/kg. Considering that these results were obtained by different authors, that the number of daily injections varied and that the injections were made either subcutaneously or intraperitoneally, whereas in the present experiments the effects of all four inhibitors were obtained in exactly comparable conditions immediately after a single intraperitoneal injection, it is not surprising that the relative potencies of the four inhibitors were not the same for the two effects. But for both effects, tranylcypromine and pheniprazine were the most potent substances and pargyline was the least potent one. This again suggests that the common mechanism for both effects is the inhibition of the brain MAO.

Reserpine which depletes the brain monoamines as first shown for noradrenaline by Holzbauer & Vogt (1956) and for 5-HT by Pletscher, Shore & Brodie (1956), is known to act by interference with the storage but not with the synthesis of 5-HT (Tozer, Neff & Brodie, 1966). If storage were completely inhibited the hypothalamus should practically be depleted of its 5-HT content. This does not happen in the conditions of the present experiments with injections of reserpine into the cerebral ventricles. Dr. D. F. Sharman and Dr. Marthe Vogt (personal communication) determined the noradrenaline and 5-HT content of the hypothalamus in cats which had been given two intraventricular injections of reserpine, each of 0·5 mg, in the same way as in the present experiments. They found that the noradrenaline was reduced to a mean of 6% but the 5-HT to between 16 and 43% (mean 27%) only. It is therefore not surprising that the 5-HT content in the effluent from the perfused cerebral ventricles was unchanged after reserpine and that tranylcypromine still increased the 5-HT output though not to the same extent.

p-Chlorophenylalanine on the other hand, interferes with the synthesis of 5-HT (Koe & Weissman, 1966). This explains why the 5-HT output from the perfused cerebral ventricles was reduced and also why the effect of tranylcypromine in increasing the 5-HT output was greatly reduced after *p*-chlorophenylalanine. No determinations have been made of the 5-HT content of the hypothalamus in cats treated with *p*-chlorophenylalanine in the same way as in the present experiments —that is 3 days after an intraperitoneal injection of 300 mg/kg. Koella, Feldstein & Czicman (1968), however, found that 3 days after an intraperitoneal injection of half the dose (150 mg/kg) the 5-HT content of the cat's diencephalon was reduced to a mean of 15%.

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REFERENCES

BANERJEE, U., FELDBERG, W. & LOTTI, V. J. (1968). Effect on body temperature of morphine and ergotamine injected into the cerebral ventricles of cats. *Br. J. Pharmac. Chemother.*, **32**, 523–538.

BRODIE, B. B., SPECTOR, S. & SHORE, P. A. (1959). Interaction of monoamine oxidase inhibition with physiological and biochemical mechanisms in brain. *Ann. N.Y. Acad. Sci.*, **80**, 609–614.

CARMICHAEL, E. A., FELDBERG, W. & FLEISCHHAUER, K. (1964). Methods for perfusing different parts of the cat's cerebral ventricles with drugs. *J. Physiol. Lond.*, **173**, 354–367.

EL HAWARY, M. B. E., FELDBERG, W. & LOTTI, V. J. (1967). Monoamine oxidase inhibition: effect on 5-HT output from the perfused third ventricle and body temperature. *J. Physiol., Lond.*, **188**, 131–140.

FUNDERBURK, W. H., FINGER, K. F., DRAKONTIDES, A. B. & SCHNEIDER, J. A. (1962). EEG and biochemical findings with MAO inhibitors. *Ann. N.Y. Acad. Sci.*, **96**, 289–302.

GOLDBERG, L. I. (1964). Monoamine oxidase inhibitors. *J. Am. med. Ass.*, **190**, 456–462.

GOLDBERG, L. I. & SJOERDSMA, A. (1959). Effects of severel monoamine oxidase inhibitors on cardiovascular actions of naturally occurring amines in dog. *J. Pharmac. exp. Ther.*, **127**, 212–218.

HOLZBAUER, M. & VOGT, M. (1956). Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.*, **1**, 8–11.

HORITA, A. (1961). The route of administration of some hydrazine compounds as a determinant of brain and liver monoamine oxidase inhibition. *Toxic. appl. Pharmac.*, **3**, 474–480.

KINROSS-WRIGHT, J. (1959). Symposium on monoamine oxidase inhibitors. *Ann. N.Y. Acad. Sci.*, **80**, 840.

KOE, B. K. & WEISSMAN, A. (1966). *p*-Chlorophenylalanine: a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.*, **154**, 499–516.

KOELLA, W. P., FELDSTEIN, A. & CZICMAN, J. S. (1968). The effect of para-chlorophenylalanine on the sleep of cats. *Electroenceph. clin. Neurophysiol.*, **25**, 481–490.

LEE, W. C., SHIN, W. H. & SHIDEMAN, F. E. (1961). Cardiac activities of several monoamine oxidase inhibitors. *J. Pharmac. exp. Ther.*, **133**, 180–185.

MERLIS, J. K. (1940). The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog. *Am. J. Physiol.*, **131**, 67–72.

PLETSCHER, A., SHORE, P. A. & BRODIE, B. B. (1956). Serotonin as a mediator of sererpine action in brain. *J. Pharmac. exp. Ther.*, **116**, 84–89.

SCHOEPKE, H. G. & WIEGAND, R. G. (1963). Relation between norepinephrine accumulation or depletion and blood pressure responses in the cat and rat following pargyline administration. *Ann. N.Y. Acad. Sci.*, **107**, 924–934.

SPENCER, J. N. (1960). Observations on cardiovascular effects of tranylcypromine. *Fedn Proc.*, **19**, 277.

TOZER, T. N., NEFF, N. H. & BRODIE, B. B. (1966). Application of steady state kinetics to the synthesis rate and turnover time of serotonin in the brain of normal and reserpine-treated rats. *J. Pharmac. exp. Ther.*, **153**, 177–182.

VANE, J. R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

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