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On the mechanism of 5-hydroxytryptamine release by thymoleptics

A. CARLSSON, J. JONASON AND M. LINDQVIST

Department of Pharmacology, University of Göteborg, Göteborg, Sweden

Rabbits were treated with nialamide followed by chlorimipramine, imipramine, desipramine or amitriptyline. Cortical brain slices were prepared and incubated with Krebs-Henseleit solution. Release of 5-hydroxytryptamine (5-HT) into the incubation medium was measured. Chlorimipramine, imipramine and amitriptyline caused release of 5-HT, whereas desipramine was without effect. The ability of cortical brain slices to retain 5-HT was dependent on energy supply, involving both aerobic and anaerobic metabolism. The data support the view that the antidepressant drugs block the membrane pump of 5-HT neurons. The observations also indicate that 5-HT is involved in thermoregulation.

Previously we have shown that the amine uptake by the membrane group of 5-hydroxytryptamine (5-HT) neurons could be blocked by tertiary amines belonging to the group of tricyclic antidepressants, for example, imipramine, chlorimipramine and amitriptyline. Chlorimipramine proved particularly potent in this respect. Secondary amines, however, such as desipramine and protriptyline, were more efficient in blocking the membrane pump of noradrenaline than of 5-HT neurons (Carlsson, Corrodi & others, 1969a,b).

In a recent paper (Carlsson, Fuxe & others, 1969) we have reported that blockade of the membrane pump of 5-HT neurons leads to potentiation of the response to monoamine oxidase (MAO) inhibitors. If chlorimipramine was given, for example, to rabbits pretreated with nialamide, shivering-like tremors with marked increase in rectal temperature occurred. When cortical slices obtained from animals treated in this manner were incubated in a Krebs-Henseleit solution a highly significant release of brain 5-HT occurred, as compared to control animals treated with nialamide only.

In the present report we extend the earlier observations with chlorimipramine to include also imipramine, desipramine and amitriptyline. Furthermore, we have investigated the problem of whether the ability of the brain tissue to retain 5-HT is dependent on energy supply.

EXPERIMENTAL

White female rabbits (1·2–2·5 kg) were treated with nialamide (100 mg/kg, i.p.) about 16 h before the administration of chlorimipramine or other antidepressant drugs. The rectal temperatures were measured before the antidepressant drug was given and immediately before death. The animals were killed by air embolism 30 min after administration of the antidepressant drug. The chest cavity was then opened and the rabbits were exsanguinated before removing the brains. Cortical slices were prepared according to McIlwain & Rodnight (1962). The slices (about 1 g) were incubated at 37° in 10 ml of Krebs-Henseleit solution* equilibrated with 5%

* Each litre of Krebs-Henseleit solution contained: NaCl, 6·89 g; KCl, 0·35 g; $CaCl_2$, 0·28 g; $MgSO_4$, 0·14 g; $EDTA.2H_2O$, 0·015 g; KH_2PO_4, 0·16 g; $NaHCO_3$, 2·0 g; glucose, 2·0 g; ascorbic acid, 0·02 g.

carbon dioxide in oxygen. In some experiments 5% carbon dioxide in nitrogen was used instead, or the glucose in the Krebs-Henseleit solution was omitted, or both.

After 40 min incubation, tissue and incubation fluid were separated by centrifugation, and both fractions were analysed for 5-HT by the method of Andén & Magnusson (1967). The release was calculated as the ratio of the 5-HT in the incubation medium to that in the incubation medium plus the brain tissue.

RESULTS

Table 1 shows the effects of imipramine, desipramine, chlorimipramine and amitriptyline when given in different doses to rabbits pretreated with nialamide (100 mg/kg, i.p. 16 h beforehand) on the 5-HT content of slices and incubation fluid, and on rectal temperature and on gross behaviour. Chlorimipramine proved to be the most potent of the drugs tested in releasing 5-HT from cortical brain slices during incubation in Krebs-Henseleit solution. The release of 5-HT after 6·25, 12·5 and 25 mg/kg, i.p. was 24, 32 and 36%, respectively, compared to 9% in controls treated with nialamide only. Amitriptyline was less active than chlorimipramine, a release of 19% being observed after both 12·5 and 25 mg/kg. Imipramine, 12·5 mg/kg,

Table 1. *5-HT content of cortical brain slices and incubation medium after incubation for 40 min in Krebs-Hensleit solution.* Rabbits were treated with nialamide alone, 100 mg/kg, i.p., 16 h beforehand (controls) or in combination with various doses of antidepressant drugs. The animals were killed 30 min after the injection of the antidepressant drug. Values (μg/g tissue) are given as means ± s.e. Figures within brackets indicate number of experiments. Shown also are the average rectal temperatures immediately before the injection of the antidepressive drug and before killing the animals.

Treatment	Dose mg/kg i.p.	Incubation medium	Brain tissue	Release % total	Initial temperature	Final temperature
Control	—	0·06 (31) ±0·007	0·59 (30) ±0·027	8·9 (30) ±0·78	38·8 (24)	38·9 (18)
Imipramine HCl	12·5	0·14 (3) ±0·024	0·54 (3) ±0·035	21·2 (3) ±3·82	38·6 (3)	39·2 (3)
	25	0·05 (3) ±0·004	0·63 (3) ±0·133	7·5 (3) ±1·51	39·3 (3)	39·5 (3)
	50	0·04 (1)	0·74 (1)	5·1 (1)	39·1 (1)	39·3 (1)
Desipramine HCl	6·25	0·06 (1)	0·73 (1)	7·6 (1)	38·5 (1)	39·3 (1)
	12·5	0·05 (1)	0·60 (1)	7·7 (1)	38·2 (1)	38·1 (1)
	25	0·06 (2) ±0·015	0·71 (2) ±0·075	7·5 (2) ±2·55	39·4 (2)	39·5 (2)
	50	0·10 (2) ±0·025	0·67 (2) ±0·130	12·3 (2) ±0·75	39·0 (2)	39·5 (2)
Chlorimipramine HCl ..	6·25	0·18 (3) ±0·075	0·53 (3) ±0·081	23·6 (3) ±7·41	39·2 (3)	40·2 (3)
	12·5	0·26 (19) ±0·030	0·55 (20) ±0·033	31·5 (19) ±2·95	39·0 (17)	40·7 (17)
	25	0·31 (3) ±0·047	0·60 (3) ±0·168	35·5 (3) ±3·42	39·1 (3)	>41·7 (3)
Amitriptyline HCl	12·5	0·17 (3) ±0·024	0·71 (3) ±0·104	18·9 (3) ±0·54	39·3 (3)	39·5 (3)
	25	0·15 (3) ±0·050	0·62 (3) ±0·096	18·6 (3) ±3·35	38·8 (3)	38·9 (3)

appeared to be as efficient as the corresponding dose of amitriptyline but at higher dose levels (25 or 50 mg/kg) no effect was found. Desipramine was ineffective in all doses investigated.

In the experiments with chlorimipramine a correlation between the effect on gross behaviour (shivering-like tremors), rise in rectal temperature and 5-HT release appeared to exist. In the individual rabbits the response varied considerably.

Amitriptyline caused less marked tremor than chlorimipramine and almost no effect on temperature. The rabbits were heavily sedated, lying on their sides.

After imipramine and desipramine, following nialamide pretreatment, the rabbits were restless and tense, but no tremor and no rise in temperature were observed.

Combination of chlorimipramine with imipramine or desipramine was also tried. The doses of imipramine and desipramine were 25 mg/kg. When imipramine was given together with chlorimipramine, 25 mg/kg, the effects on behaviour, temperature and 5-HT release were about the same as after chlorimipramine alone. Combination of chlorimipramine, 12·5 mg/kg, with desipramine caused only a slight rise in temperature, and with imipramine the release of 5-HT seemed to be lower than that after chlorimipramine alone (Table 2).

Table 2. *Effect of combined treatment with chlorimipramine and imipramine or desipramine on 5-HT content of cortical brain slices and incubation medium after incubation for 40 min in Krebs-Hensleit solution.* Rabbits were treated with nialamide, 100 mg/kg 16 h beforehand, followed by chlorimipramine alone or in combination with imipramine or desipramine. The chlorimipramine plus imipramine or desipramine were injected simultaneously and the animals were killed after 30 min. For further explanations see Table 1.

	Incubation medium	Brain tissue	Release % total	Initial temperature	Final temperature
Chlorimipramine 12·5 mg/kg	0·26 (19) ±0·030	0·55 (20) ±0·033	31·5 (19) ±2·95	39·0 (17)	40·7 (17)
Chlorimipramine 25 mg/kg	0·31 (3) ±0·047	0·60 (3) ±0·168	35·5 (3) ±3·42	39·1 (3)	>41·7 (3)
Imipramine 25 mg/kg + chlorimipramine 12·5 mg/kg	0·13 (3) ±0·063	0·62 (3) ±0·080	17·2 (3) ±8·99	39·2 (3)	39·7 (3)
Imipramine 25 mg/kg + chlorimipramine 25 mg/kg	0·32 (3) ±0·075	0·46 (3) ±0·092	40·1 (3) ±1·33	39·5 (3)	40·9 (3)
Desipramine 25 mg/kg + chlorimipramine 12·5 mg/kg	0·17 (3) ±0·067	0·51 (3) ±0·049	22·9 (3) ±6·58	38·8 (3)	39·1 (3)

The ability of the brain tissue to retain 5-HT seemed to be dependent on energy supply. In experiments with rabbits treated with nialamide alone, cortical slices were incubated in glucose-free Krebs-Henseleit solution under an atmosphere of N_2 and CO_2. The release of 5-HT increased to about 70% compared to about 9% if the incubation was performed with glucose under O_2 and CO_2 (Table 3). If either glucose was omitted or oxygen replaced by nitrogen, intermediate release values were obtained.

Chlorimipramine given to rabbits pretreated with nialamide caused a release of about 35% in the presence of glucose and oxygen. If both glucose and oxygen were omitted (i.e. oxygen replaced by nitrogen) the release of 5-HT was about the same as after nialamide alone under these conditions. If either of the two was omitted intermediate values were obtained.

Table 3. *Effect of glucose deprivation and/or anoxia on the 5-HT content of cortical brain slices and incubation medium after incubation for 40 min in Krebs-Henseleit solution.* Rabbits were treated with nialamide alone or in combination with chlorimipramine. The animals were killed 30 min after injection of chlorimipramine. Single values are given in μg/g tissue. Shown also are rectal temperatures immediately before the injection of chlorimipramine and before killing the animals. The degrees of behavioural effect of chlorimipramine is indicated by an arbitrary scale ranging from O (no effect) to ++++ (very strong effect).

	0·2% glucose + oxygen			No glucose + oxygen			0·2% glucose + nitrogen			No glucose + nitrogen					
	Incubation medium	Brain tissue	Release % total	Incubation medium	Brain tissue	Release % total	Incubation medium	Brain tissue	Release % total	Incubation medium	Brain tissue	Release % total	Initial temperature	Final temperature	Effect on behaviour
Nialamide	0·40	0·58	40·8	0·41	0·33	55·4	—	—	—	—	—	—	39·0	40·5	+++
100 mg/kg i.p.	0·09	0·91	9·0	0·21	0·37	36·2	—	—	—	—	—	—	38·8	38·8	O
+	0·23	0·32	41·8	0·33	0·35	48·5	—	—	—	—	—	—	38·6	41·0	+++
Chlorimipramine	0·37	0·54	40·7	—	—	—	0·33	0·39	45·8	—	—	—	38·8	42·0	++++
12·5 mg/kg i.p.	0·41	0·57	41·8	—	—	—	0·40	0·37	51·9	—	—	—	38·9	40·5	++++
	0·25	0·42	37·3	—	—	—	0·23	0·39	37·1	—	—	—	38·7	40·6	+
	0·06	0·55	9·8	—	—	—	—	—	—	0·44	0·15	74·6	39·2	39·3	+
	0·47	0·45	51·1	—	—	—	—	—	—	0·72	0·29	71·3	38·8	41·3	++++
	—	0·45	—	—	—	—	—	—	—	0·32	0·10	76·2	39·7	38·5	+
Mean	0·29	0·53	34·0	0·32	0·35	46·7	0·32	0·38	44·9	0·49	0·18	74·0	38·9	40·3	
± s.e.	±0·054	±0·055	±5·55	±0·058	±0·012	±5·62	±0·049	±0·007	±4·29	±0·119	±0·056	±1·44			
Nialamide	0·02	0·64	3·0	0·18	0·50	26·5	—	—	—	—	—	—	39·0	38·8	
100 mg/kg i.p.	0·06	0·51	10·5	0·15	0·29	34·1	—	—	—	—	—	—	39·0	39·0	
	0·03	0·68	4·2	0·04	0·57	6·6	—	—	—	—	—	—	38·7	38·7	
	0·05	0·59	7·8	0·11	0·47	19·0	—	—	—	—	—	—	38·4	—	
	0·06	0·62	8·8	—	—	—	0·19	0·53	26·4	—	—	—	38·8	38·8	
	0·18	0·81	18·2	—	—	—	0·45	0·57	44·1	—	—	—	39·3	39·6	
	0·05	0·54	8·5	—	—	—	0·17	0·37	31·5	—	—	—	—	—	
	0·05	0·61	7·6	—	—	—	—	—	—	0·58	0·18	76·3	38·8	38·8	
	0·08	—	—	—	—	—	—	—	—	—	0·20	—	38·5	38·5	
	0·06	0·52	10·3	—	—	—	—	—	—	0·54	0·22	71·1	39·2	39·2	
	0·06	0·64	8·6	—	—	—	—	—	—	0·44	0·21	67·7	39·0	39·0	
	0·08	0·71	10·1	—	—	—	—	—	—	0·56	0·26	68·3	38·6	—	
Mean	0·07	0·62	8·9	0·12	0·46	21·6	0·27	0·49	34·0	0·53	0·21	70·9	38·8	38·9	
± s.e.	±0·012	±0·027	±1·18	±0·030	±0·060	±5·86	±0·090	±0·061	±5·26	±0·031	±0·013	±1·96	—	—	

DISCUSSION

Imipramine and amitriptyline treatment of nialamide pretreated rabbits caused a moderate increase in the release of 5-HT from cortical brain slices *in vitro*. In this respect these drugs were less potent than chlorimipramine, whereas desipramine had no effect. This order of activity corresponds to our earlier observations on 5-hydroxytryptophan potentiation and the ability to block the membrane pumps of 5-HT neurons, using 5-HT depletion by 4-methyl-α-ethyl-*m*-tyramine (H75/12) as indicator. It therefore seems reasonable to assume that the 5-HT release induced by antidepressant drugs, as observed in the present investigation, is due to blockade of the membrane pump of 5-HT neurons, unmasking a rather pronounced leakage of 5-HT following inhibition of monoamine oxidase. In support of this assumption it was observed that the ability of cortical tissue to retain 5-HT was dependent on energy supply. Moreover, deprivation of energy supply eliminated the 5-HT releasing action of chlorimipramine.

To obtain a maximum 5-HT release it was necessary to deprive the tissue of both oxygen and glucose. The data indicate that the membrane pump of 5-HT neurons can derive its energy supply both from aerobic metabolism and anaerobic glycolysis.

The correlation between 5-HT release and rise in body temperature observed in the present investigation supports the hypothesis of Feldberg & Myers (Feldberg & Myers, 1963; Feldberg, 1968; cf. Corrodi, Fuxe & Hökfelt, 1967) that 5-HT is involved in thermoregulation.

Our results suggest a certain degree of antagonism between chlorimipramine and imipramine/desipramine as regards 5-HT release and rise in temperature. Further work is needed to establish the mechanism involved. It is tempting to speculate on an interaction between 5-HT and noradrenaline neurons. Such an interaction might also explain the apparently dual action of imipramine, indicated by an irregular dose-response relationship.

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