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Effects of 5-hydroxytryptamine, fluoxetine and chlorimipramine on reflex bradycardia in rats

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In the urethane-anaesthetized rat, increasing 5-hydroxytryptaminergic receptor activity or functional 5-HT in brain with either the specific inhibitors of 5-HT reuptake (e.g. fluoxetine and chlorimipramine) or the 5-HT itself greatly reduced the reflex bradycardia induced by the systemically administered adrenaline. The data indicate that activation of central 5-HT-ergic receptors tends to inhibit reflex bradycardia.

Recently, the effects of brain 5-hydroxytryptamine (5-HT) alterations on reflex bradycardia in rats have been assessed in our laboratory (Lin & Chern 1979). Elevation of the 5-HT content in brain with 5-hydroxytryptophan after peripheral decarboxylase inhibition with benserazide produced a significant reduction in reflex bradycardia compared with the controls. In contrast, depletion of the 5-HT content in brain with either *p*-chlorophenylalanine (PCPA) or 5,7-dihydroxytryptamine led to an enhancement of adrenaline-induced bradycardia. Moreover, the enhanced bradycardia induced by PCPA treatment was readily blocked by the replacement of the depleted brain 5-HT with 5-hydroxytryptophan and benserazide. The results suggested that brain 5-HT systems appear to be an inhibitory neural pathway for the development of reflex bradycardia.

In the present study, the effects of exogenous administration of 5-HT and the specific inhibitors of 5-HT reuptake such as fluoxetine and chlorimipramine on adrenaline-induced bradycardia were assessed in rats, in order to test the possibility.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 250 and 300 g were anaesthetized with either urethane (300 mg kg^{-1} i.p.) or sodium pentobarbitone (60 mg kg^{-1} i.p.). Before experiments they were housed individually in wire-mesh cages in a room at 25 ± 1·0 °C with a natural light-dark cycle with free access to tap water and granular chicken feed.

Experimental design. Four groups of animals were used: (1) controls receiving either an intraventricular (i. vent) or an i.v. dose of 0·9% NaCl (saline); (2) rats receiving either an i. vent. or i.v. dose of fluoxetine; (3) rats receiving either an i. vent. or i.v. dose of chlorimipramine; and (4) rats receiving an i. vent. dose of 5-HT. Those treated with fluoxetine and chlorimipramine i. vent. were studied 30 min after injection, those treated i.p. were studied 60 min after injection. Rats given 5-HT i. vent. were studied 10 min after injection. The vasopressor and bradycardiac responses of the anaesthetized animals to intravenous doses of adrenaline were observed.

Surgical techniques. Each rat was anaesthetized intraperitoneally with urethane, and a burr hole was drilled in the calvarium above the left cerebral ventricle. The stereotaxic co-ordinates employed for the intraventricular injection were derived from the atlas of DeGroot (DeGroot 1959) at AP, 4·8; L, 2·5; and H, 3·0. The position of the cannula was considered correct if 5 μl of saline or of the drug solutions flowed in by gravity over 20 s.

Physiological measurements. Rectal temperature was maintained at 37·5 ± 0·5 °C throughout the experiments by infrared light. The right femoral artery was catheterized. The femoral arterial pressure was monitored with a Statham P23AC transducer and heart rate with a Grass 7C tachometer triggered by arterial pulses. The right femoral vein was cannulated for intravenous injection. All recordings were made on a four-channel Grass 7C polygraph.

Drug solutions. These were prepared in pyrogen-free glassware which was baked at 180 °C for 5 h before use. Drugs administered intraperitoneally included adrenaline U.S.P. (Retired Servicemen's Pharmaceutical Plant of Taiwan, 1–5 μg kg^{-1}); fluoxetine (Lilly 110140, donated by Lilly & Co., Indianapolis, Ind., 5 mg kg^{-1}); and chlorimipramine (donated by Ciba Co. 1 mg kg^{-1}). Drugs administered intraventricularly included fluoxetine (50–100 μg), chlorimipramine (100–150 μg) and 5-HT

* Correspondence.

(20–100 μg). For the injection of drugs into the brain, the injection techniques were as described by Lin (1978a, 1979).

RESULTS

The reflex bradycardia was produced by intravenous administration of adrenaline in rats. Over the dose range (1–5 μg kg^{-1}) of adrenaline, a dose-dependent bradycardia was obtained. The dose (2·5 μg kg^{-1}) was chosen for the following experiments.

Table 1 summarizes the vasopressor and bradycardiac response to an intravenous dose of adrenaline in control animals and in the urethane-anaesthetized animals treated with fluoxetine or chlorimipramine i.v. In the saline-controlled rats adrenaline produced a decrease in heart rate and an increase in mean arterial pressure. In the drug-treated rats, the bradycardiac responses were attenuated significantly, although the responses of mean arterial pressure were not significantly different from controls. In addition, in rats under sodium pentobarbitone anaesthesia, the bradycardia induced by adrenaline was also reduced by pretreatment with an i.v. dose of fluoxetine or chlorimipramine (Table 2).

Table 3 summarizes the effects of intraventricular administration of 5-HT, fluoxetine and chlorimipramine on adrenaline-induced bradycardia in urethane-anaesthetized rats. The bradycardia induced by adrenaline was attenuated greatly by pretreatment with 5-HT, fluoxetine or chlorimipramine. There were no changes in the arterial pressure responses induced by adrenaline.

There were no significant effects of any of the drug treatments on the basal levels of both mean arterial pressure and heart rate.

Table 1. Effects of intravenous administration of fluoxetine and chlorimipramine on the vasopressor and bradycardiac responses induced by an i.v. dose of adrenaline in urethane-anaesthetized rats.

	Mean arterial pressure, Torr.			Heart rate, beats min^{-1}		
Treatment	Control	After adrenaline	Difference	Control	After adrenaline	Difference
1. Saline + adrenaline 1 μg kg^{-1}, n = 8	105 ± 19·6	145 ± 24·7	40 ± 12·3	411 ± 21·4	370 ± 17·9	−41 ± 6·7
2. Saline + adrenaline 2·5 μg kg^{-1}, n = 8	108 ± 16·8	176 ± 25·4	68 ± 12·7	412 ± 25·6	328 ± 17·2	−82 ± 9·5
3. Saline + adrenaline 5 μg kg^{-1}, n = 8	110 ± 20·8	204 ± 28·1	94 ± 9·8	406 ± 21·5	274 ± 19·5	−132 ± 14·3
4. Fluoxetine 5 mg kg^{-1} + adrenaline 2·5 μg kg^{-1}, n = 8	104 ± 18·2	164 ± 17·6	60 ± 7·2	410 ± 23·5	379 ± 14·7	−31 ± 6·4*
5. Chlorimipramine 1 mg kg^{-1} + adrenaline 2·5 μg kg^{-1}, n = 8	107 ± 12·4	177 ± 16·9	70 ± 9·7	405 ± 19·6	365 ± 15·7	−40 ± 9·3*

* Significantly different from corresponding control value (saline + adrenaline 2·5 μg kg^{-1}), $P < 0·05$ (one way analysis of variance). The values are expressed as the mean ± s.e.m. n, number of rats tested.

Table 2. Effects of intravenous administration of fluoxetine and chlorimipramine on the vasopressor and bradycardiac responses induced by an i.v. dose of adrenaline in sodium pentobarbitone-anaesthetized rats.

	Mean arterial pressure, Torr.			Heart rate, beats min^{-1}		
Treatment	Control	After adrenaline	Difference	Control	After adrenaline	Difference
1. Saline + adrenaline 2·5 μg kg^{-1}, n = 6	99 ± 14·2	164 ± 22·6	65 ± 9·2	395 ± 19·8	311 ± 16·6	−84 ± 8·7
2. Fluoxetine 5 mg kg^{-1} + adrenaline 2·5 μg kg^{-1}, n = 6	96 ± 13·8	156 ± 20·3	60 ± 8·5	390 ± 18·7	348 ± 20·2	−42 ± 9·7*
3. Chlorimipramine 1 mg kg^{-1} + adrenaline 2·5 μg kg^{-1}, n = 6	92 ± 12·5	154 ± 19·8	62 ± 7·7	387 ± 22·4	342 ± 22·4	−45 ± 7·3*

* Significantly different from corresponding control value (saline + adrenaline), $P < 0·05$ (one way analysis of variance). The values are expressed as the mean ± s.e.m. n, number of rats tested.

Table 3. Effects of intraventricular administration of 5-HT, fluoxetine and chlorimipramine on the vasopressor and bradycardiac responses induced by an i.v. dose of adrenaline in urethane-anaesthetized rats.

Treatment	Mean arterial pressure, Torr.			Heart rate, beats min^{-1}		
	Control	After adrenaline	Difference	Control	After adrenaline	Difference
1. Saline + adrenaline 2·5 $\mu g\ kg^{-1}$, n = 8	106 ± 15·3	174 ± 24·2	68 ± 11·4	409 ± 24·7	329 ± 15·6	−80 ± 8·8
2. 5-HT 20 μg + adrenaline 2·5 $\mu g\ kg^{-1}$, n = 8	105 ± 13·9	178 ± 23·1	73 ± 9·8	408 ± 23·6	357 ± 15·3	−51 ± 7·5*
3. 5-HT 50 μg + adrenaline 2·5 $\mu g\ kg^{-1}$, n = 8	107 ± 14·6	177 ± 22·3	70 ± 9·6	410 ± 25·7	380 ± 16·4	−30 ± 6·2*
4. 5-HT 100 μg + adrenaline 2·5 $\mu g\ kg^{-1}$, n = 8	104 ± 12·8	170 ± 24·4	66 ± 10·7	405 ± 26·3	393 ± 17·7	−12 ± 4·2*
5. Fluoxetine 50 μg + adrenaline 2·5 $\mu g\ kg^{-1}$, n = 8	103 ± 13·5	172 ± 21·7	69 ± 8·8	405 ± 22·8	365 ± 14·2	−40 ± 7·1*
6. Fluoxetine 100 μg + adrenaline 2·5 $\mu g\ kg^{-1}$, n = 8	107 ± 14·9	175 ± 22·5	68 ± 11·3	407 ± 23·4	392 ± 15·5	−15 ± 5·8*
7. Chlorimipramine 100 μg + adrenaline 2·5 $\mu g\ kg^{-1}$, n = 8	110 ± 14·8	175 ± 19·3	65 ± 8·7	411 ± 20·4	374 ± 14·8	−37 ± 6·6*
8. Chlorimipramine 150 μg + adrenaline 2·5 $\mu g\ kg^{-1}$, n = 8	108 ± 12·9	174 ± 20·1	66 ± 9·7	410 ± 21·5	381 ± 16·5	−19 ± 5·7*

* Significantly different from corresponding control value, P value $<0·05$ (one way analysis of variance). The values are expressed as the mean ± s.e.m. n, number of rats tested.

DISCUSSION

It is well known that a relatively small number of 5-HT cell bodies in the pons and medulla oblongata give rise to substantial ascending and descending fibre tracts. Recently, it has been found that sinoaortic denervation caused a selective increase in endogenous concentrations of 5-HT in the medulla, pons and the thoracolumbar region of the spinal cord; no significant changes occurred in the six other areas of the brain and spinal cord were examined (Wing & Chalmers 1973). These results are consistent with an increase in the activity of bulbospinal 5-HT-ergic nerves in neurogenic hypertension. Moreover, depletion of 5-HT in the spinal cord with intracisternal administration of 5,6-dihydroxytryptamine has been shown to prevent the increase in arterial blood pressure and heart rate produced by sinoaortic denervation and to reverse them when they are already established (Wing & Chalmers 1973, 1974). In addition, our recent results showed that elevating the brain content of 5-HT depressed adrenaline-induced bradycardia, whereas depleting brain 5-HT enhanced the induced bradycardia (Lin & Chern 1979). These observations suggested that brain 5-HT may be an inhibitory neurotransmitter for the development of reflex bradycardia.

The hypothesis is substantiated by the present results which show that exogenous administration of either 5-HT itself or the specific inhibitors of 5-HT reuptake, such as fluoxetine and chlorimipramine, produced important reflex bradycardia inhibition in urethane-anaesthetized rats. Either the inhibition of 5-HT reuptake or the exogenous administration of 5-HT would result in an increased level of 5-HT available to the 5-HT-ergic receptors (Carlsson et al 1969; Wong et al 1974; Lin 1978b). Thus the data indicate that any increase in 5-HT receptor activity or in functional 5-HT in the brain tends to inhibit reflex bradycardia.

However, it must be acknowledged that the drugs used in the present experiments exhibited a variety of non-specific effects in addition to altering transmission in 5-HT pathways within brain. For example, the inhibitors of 5-HT reuptake, fluoxetine and chlorimipramine, have been shown to decrease 5-HT turnover or the firing rate of raphe neurons (Bramwell 1972; Fuller et al 1974). Furthermore, leakage may occur from intracerebroventricular injections of drugs into the peripheral circulation and may produce a non-specific action at a cardiac level. Such side effects cannot be completely ruled out in the present study as mediating the observed

inhibition in reflex bradycardia. Moreover, the choice of urethane as the anaesthetic causes a massive increase in autonomic discharge, a marked rise in circulating catecholamines and hyperglycaemia. Thus the observed bradycardia is dependent upon the balance between the direct positive chronotropic actions of adrenaline and the extent of reflex vagal inhibition. However, the present results did show that, in rats under sodium pentobarbitone anaesthesia, the bradycardia induced by adrenaline was greatly reduced by increasing 5-HT receptor activity in the brain. It is therefore unlikely that the present results reflect only an experimental artifact particular to urethane anaesthetized rats.

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