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Effect of lofepramine and other antidepressants on the uptake of 5-hydroxytryptamine and noradrenaline into rat brain monoaminergic neurons

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Lofepramine, (*N*-methyl-*N*-[4-chlorobenzoylmethyl]-3-[10, 11-dihydro-5H-dibenz(b,f)-azepin-5-yl]-propylamine hydrochloride), is a new antidepressant with low toxicity and no peripheral anticholinergic activity. Its effect on 5-hydroxytryptamine (5-HT) and noradrenaline uptake into rat brain monoaminergic neurons was studied and compared with that of other antidepressants, particularly with that of imipramine and desipramine. Lofepramine inhibited both 5-HT and noradrenaline uptake into synaptosomal fractions *in vitro* but was 4 times more potent in inhibiting noradrenaline than 5-HT uptake, indicating the effect resembles that of desipramine. Noradrenaline uptake was also preferentially inhibited in synaptosomes from brain of rats treated previously with lofepramine or desipramine (i.p.). Pretreatment with SKF 525A (i.p.) did not diminish the effect of lofepramine but rather potentiated it. Therefore it is suggested that the formation of desipramine is not necessary for lofepramine to exhibit the effect on amine uptake *in vivo*. Both lofepramine and desipramine inhibited intraventricular noradrenaline uptake into synaptosomes without any effect on 5-HT uptake. These results suggest that lofepramine is qualitatively similar to desipramine with respect to preferential inhibition of noradrenaline uptake into central noradrenergic neurons.

Lofepramine, *N*-methyl-*N*-[4-chlorobenzoylmethyl]-3-[10,11-dihydro-5H-dibenz(b, f)azepin-5-yl]-propylamine hydrochloride is a tertiary amine and an imipramine analogue with one of the *N*-methyl groups substituted by a 4′-chlorophenacyl group. This makes it much more lipophilic and less basic than imipramine or its derivative desipramine (Eriksoo & Rohte, 1970).

Imipramine and related tertiary amines inhibit 5-hydroxytryptamine (5-HT) uptake by serotoninergic neurons while secondary amines like desipramine preferentially inhibit noradrenaline uptake by catecholaminergic neurons (Fuxe & Ungerstedt, 1967; Carlsson, Fuxe & Ungerstedt, 1968; Fuxe & Ungerstedt, 1968; Segawa & Fujisawa, 1972). We have compared the effect of lofepramine with that of other antidepressants on 5-HT and noradrenaline uptake.

MATERIALS AND METHODS

Materials

[^{3}H]-5-HT creatinine sulphate (^{3}H-5-HT) (0·5 Ci mmol^{-1}) was obtained from The Radiochemical Centre, Amersham and [7-^{3}H]-(—)-noradrenaline (^{3}H-NA) (3·846 or 10·3 Ci mmol^{-1}) from New England Nuclear Corporation. The following drugs were generously donated: lofepramine hydrochloride, imipramine hydrochloride, amitriptyline hydrochloride (Daiichi Pharmaceutical Co. Ltd., Tokyo); desipramine hydrochloride (Fujisawa Pharmaceutical Co. Ltd., Osaka); SKF 525A (Smith Kline & French Philadelphia); pheniprazine hydrochloride (Chugai Pharmaceutical Co. Ltd., Tokyo).

Methods

In vitro *effect of antidepressants on the uptake of 5-HT and noradrenaline into synaptosomal S_1-fraction.* Male Wistar strain rats (150–200 g) were decapitated and the whole brain (excluding cerebellum) was rapidly removed and dissected. The tissue was homogenized with 10 vol of ice-cold 0·32 M sucrose in a homogenizer with a Teflon pestle driven mechanically at 1200 rev min^{-1} (clearance 0·25 mm). The homogenates were centrifuged at 4° for 10 min at 900 *g*. The pellet was washed twice with 0·32 M sucrose and the washings were added to the supernatant fluid hereafter referred to as the S_1-fraction. Uptake of ^{3}H-5-HT or ^{3}H-NA into the S_1-fraction was by the modified method of Nomura, Tanaka & Segawa (1975) and Nomura, Naitoh & Segawa (1976). Radioactivity in the fraction was determined in a model 3320 Packard Tri-Carb scintillation

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spectrometer and corrected for efficiency by external standardization. The extent of active uptake was expressed as the difference of the values at 37° and 0°.

Amine uptake into synaptosomal S_1-fraction from intact and antidepressants-treated rats. The synaptosomal S_1-fraction was prepared from the brain of rats treated with antidepressants (i.p.) 1 h before death. The *in vitro* uptake of 5-HT or noradrenaline into this fraction was compared with that into S_1-fraction from intact animals.

Effect of intraperitoneal injection of antidepressants on the uptake of intraventricularly administered 5-HT or noradrenaline into brain. A permanent cannula was implanted into the rat (male Wistar strain, 250–280 g) brain according to Hayden, Johson & Maickel (1966) with some modification. 5-HT and noradrenaline dissolved in 0·015 ml of saline containing 0·02% ascorbate were injected intraventricularly in normal and antidepressant pretreated (i.p.) rats in a dose of 0·75 μCi and 1·2 μCi, respectively. After injection the rats were killed and the whole brain (excluding cerebellum) was fractionated. The S_1-fraction was then centrifuged at 11 500 *g* for 20 min to produce a pellet (P_2) and a supernatant (S_2).

^{3}H-5-HT in P_2- and S_2-fractions was extracted by the method of Curzon & Green (1970). ^{3}H-NA in the P_2-fraction was extracted by a modification of the method of Anton & Sayre (1962). The radioactivity in 0·5 ml of HCl was determined in 10 ml of Bray's solution as described above.

RESULTS

Table 1 shows IC50s of antidepressants required for 50% inhibition of the uptake of 5-HT and noradrenaline into synaptosomal S_1-fraction *in vitro*.

Table 1. In vitro *inhibition by antidepressants of ^{3}H-5-HT and ^{3}H-NA uptake into rat brain synaptosomes.*

Antidepressants	IC50 (M) ^{3}H-5-HT	IC50 (M) ^{3}H-NA	Ratio (5-HT/NA)
Imipramine	$3{\cdot}2(3{\cdot}0-4{\cdot}1)\times10^{-7}$	$3{\cdot}7(3{\cdot}0-5{\cdot}5)\times10^{-6}$	0·086
Amitriptyline	$2{\cdot}1(1{\cdot}6-2{\cdot}4)\times10^{-7}$	$3{\cdot}8(3{\cdot}0-5{\cdot}5)\times10^{-6}$	0·055
Desipramine	$1{\cdot}5(1{\cdot}2-2{\cdot}2)\times10^{-6}$	$3{\cdot}5(2{\cdot}0-4{\cdot}1)\times10^{-8}$	42·86
Lofepramine	$1{\cdot}1(1{\cdot}0-1{\cdot}2)\times10^{-5}$	$2{\cdot}7(1{\cdot}9-3{\cdot}4)\times10^{-6}$	4·074

Portions of the S_1-fraction were incubated with antidepressants and pheniprazine (2×10^{-5}M) for 10 min after which ^{3}H-5-HT (4×10^{-8} M) or ^{3}H-NA ($2{\cdot}6\times10^{-8}$M) was added, and incubation was continued for 2 min with ^{3}H-5-HT and 5 min with ^{3}H-NA. IC50 values were derived from log percent plots of inhibition at 3 concentrations of antidepressants ranging from 10^{-8} to 10^{-4}M and represent the mean and the range of 3 or 4 determinations. Ratio was calculated from the mean values.

The most potent inhibitor of 5-HT uptake appeared to be amitriptyline, followed by imipramine and desipramine and lofepramine. In contrast, the rank order of potency for inhibiting noradrenaline uptake was desipramine, lofepramine, imipramine and amitriptyline. Lofepramine inhibited both 5-HT and noradrenaline uptake but was 4 times more potent in inhibiting noradrenaline uptake than 5-HT uptake.

The influence of the antidepressants (i.p.) for 1 h to rats on the subsequent *in vitro* uptake of amines into the S_1-fraction is shown in Fig. 1. Injection of 23 mg kg^{-1} (i.p.) of imipramine inhibited the uptake of 5-HT by more than 60%, whereas no significant inhibition of noradrenaline uptake was observed. When rats were pretreated with 30 mg kg^{-1} (i.p.) of SKF 525A 30 min before injection (i.p.) of imipramine the effect of imipramine was significantly increased (Fig. 1). Thus, the uptake of noradrenaline was also significantly reduced but the relative selectivity of imipramine for 5-HT was not altered. Desipramine, at 20 mg kg^{-1} (i.p.) inhibited the uptake of both 5-HT and noradrenaline but at the dose used, produced a much greater inhibition of noradrenaline uptake. Injection of lofepramine, at 28 mg kg^{-1} (i.p.) did not influence the uptake of either 5-HT or noradrenaline in normal rats, but significantly decreased the uptake of both in rats pretreated with SKF 525A. Also, 45 mg kg^{-1} of

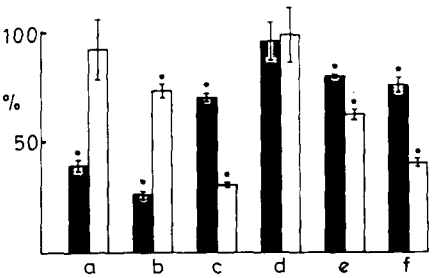

FIG. 1. Rats were injected (i.p.) with antidepressants 1 h before experiment. a—imipramine (23 mg kg^{-1}), c—desipramine (20 mg kg^{-1}), d—lofepramine (28 mg kg^{-1}), f—lofepramine (45 mg kg^{-1}). In some experiments SKF 525A (30 mg kg^{-1}) was injected 30 min before, b—imipramine (23 mg kg^{-1}) or e—lofepramine (28 mg kg^{-1}). S_1-fractions from control or antidepressant pretreated rat brains were incubated with pheniprazine (2×10^{-5}M) for 10 min after which ^{3}H-5-HT (4×10^{-8}M) or ^{3}H-NA ($2{\cdot}6\times10^{-8}$M) was added, and the incubations were continued for 2 min for 5-HT and 5 min for noradrenaline. Data are expressed as percent of control values for non-treated animals. Values are mean % inhibition of 2 to 6 determinations, vertical lines indicate s.e. of mean. *Significantly different from control (C) at $P<0{\cdot}001$. Open columns —^{3}H-NA, closed columns—^{3}H-5-HT.

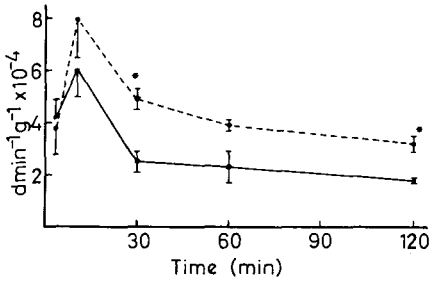

FIG. 2. Imipramine (30 mg kg^{-1}) was injected (i.p.) 30 min before the intraventricular administration of ^{3}H-5-HT (0·75 μCi). The radioactivity was determined in the synaptosomal P_2-fraction. Values are the means of 2 to 5 determinations, vertical lines indicate s.e. of mean. *Significantly different from control at $P<0·05$. ●—● Control, ● - - ● imipramine. Ordinate—^{3}H-5-HT in the P_2-fraction (d min^{-1} g^{-1} × 10^{-4}).

lofepramine decreased significantly both 5-HT and noradrenaline uptake in intact rats.

Fig. 2 shows the time course of uptake of intraventricularly injected (i. vent.) ^{3}H-5-HT into the P_2-fraction from normal and imipramine-treated rat brain. Initial rate of uptake was very rapid. Thus, in normal rats 3 min after 5-HT administration the amount of 5-HT was approximately 70% of the highest value at 10 min. A rapid decrease was observed between 10 to 30 min, thereafter continuous slow drop was observed by 120 min. Injection of 30 mg kg^{-1} (i.p.) of imipramine 30 min before intraventricular injection of 5-HT inhibited the uptake of 5-HT into the P_2-fraction at 3 min after 5-HT administration. However, rats treated with imipramine (i.p.) and killed at 10, 30, 60 and 120 min

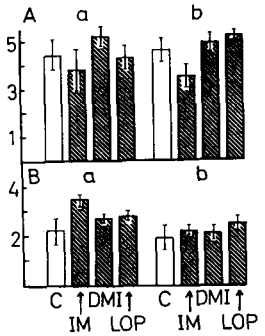

FIG. 3. Imipramine (IM) or desipramine (DMI) (30 mg kg^{-1}) or lofepramine (LOP) (50 mg kg^{-1}) was injected (i.p.) 30 min before intraventricular administration of ^{3}H-5-HT (0·75 μCi). A—3 or B—60 min after intraventricular administration of ^{3}H-5-HT the radioactivity (d min^{-1} g^{-1} × 10^{-4}) in a—P_2- and b—S_2-fractions was determined. Values are the means of 3 to 5 determinations, vertical lines indicate s.e. of mean. C—control. Ordinate—d min^{-1}g^{-1} × 10^{-4}.

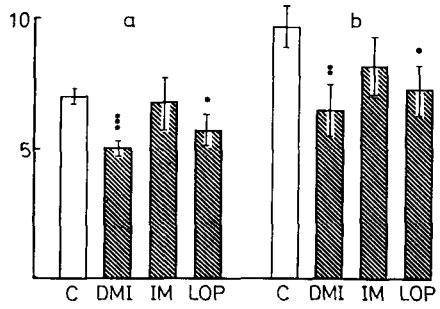

FIG. 4. Imipramine (IM) or desipramine (DMI) (30 mg kg^{-1}) or lofepramine (LOP) (50 mg kg^{-1}) was injected (i.p.) 30 min before intraventricular administration of ^{3}H-NA (1·2 μCi). 90 min after intraventricular administration of ^{3}H-NA the radioactivity (d min^{-1} g^{-1} × 10^{-4}) a—P_2- and b—S_2-fractions was determined. Values are the means of 4 to 8 determinations. Significantly different from control (C) at *$P<0·1$. **$P<0·05$. ***$P<0·01$. C—Control. Ordinate—d min^{-1}g^{-1} × 10^{-4}.

after 5-HT had higher concentrations of 5-HT in the fraction than did the controls. The time course of 5-HT uptake into the S_2-fraction was similar to that into the P_2-fraction. Pretreatment with imipramine (i.p.) inhibited the uptake of 5-HT into the S_2-fraction at 3 min but rather increased it at 60 min (Fig. 3).

The effect of desipramine or lofepramine (i.p.) is also shown in Fig. 3. At 3 min after intraventricular injection of 5-HT the 5-HT content in both the P_2- and S_2-fraction from antidepressant-treated rats was almost the same as, or slightly higher than, those in the fractions from control groups, whereas 5-HT content in both fractions increased slightly at 60 min.

Fig. 4 shows the effect of antidepressants (i.p.) on the uptake of intraventricular noradrenaline. When rats were treated with desipramine (30 mg kg^{-1}, i.p.) 30 min before intraventricular injection of noradrenaline and killed 90 min later, the uptake of noradrenaline into both the P_2- and S_2-fractions was significantly inhibited. Lofepramine was also effective in inhibiting noradrenaline uptake into both fractions at 50 mg kg^{-1}, whereas 30 mg kg^{-1} of imipramine did not influence the uptake significantly.

DISCUSSION

Kuhar (1973) reported that the maximum concentrations of 5-HT and noradrenaline for selective uptake from medium into rat forebrain synaptosomes were 10^{-7} and 5×10^{-7} M, respectively. In our previous experiment (unpublished) the Km values for the high affinity uptake of 5-HT and noradrenaline into rat brain synaptosomes were $1·7 \times 10^{-7}$ and 3×10^{-7} M, respectively. Since the medium concentrations used in this experiment were 4×10^{-8} M for 5-HT and $2·6 \times 10^{-8}$ M for noradrenaline it would seem

reasonable to assume that both amines are taken up specifically into the respective synaptosomes.

In vitro, 5-HT uptake was preferentially inhibited by amitriptyline and imipramine while noradrenaline uptake was more readily inhibited by desipramine and lofepramine.

Further experiments were made to see if these differences in sensitivity of 5-HT and noradrenaline uptake to drugs also occurred in synaptosomal fractions from brain of the rats treated previously with antidepressants (i.p.). Again imipramine inhibited 5-HT uptake preferentially while desipramine and lofepramine preferentially inhibited noradrenaline uptake. Therefore, the results closely parallel those obtained *in vitro*. The mechanism by which pretreatment with SKF 525A potentiated the effect of imipramine and lofepramine is not known although it does delay the demethylation of imipramine in rats (Bickel & Weder, 1968).

Aghajanian & Bloom (1967a, 1967b) reported that ^{3}H-5-HT and (±)-^{3}H-NA given intraventricularly were efficiently taken up by the areas that are rich in the endogenous 5-HT and noradrenaline, respectively. In the present investigation, the amounts of these amines were smaller than those used in their experiment. Furthermore (—)-^{3}H-NA might be expected to be taken up more specifically into noradrenaline neurons than (±)-^{3}H-NA. Therefore it is highly probable that in the present experiments ^{3}H-NA and ^{3}H-5-HT were taken up by adrenergic and serotoninergic neurons, respectively.

^{3}H-5-HT injected into lateral ventricle and taken up into synaptosomes decreased gradually from 10 min after injection. This is probably due to spontaneous release from nerve-endings and metabolic degradation. Therefore the 5-HT content in the P_2-fraction from imipramine-treated rat brain at 3 min after 5-HT intraventricularly is due to uptake inhibition by imipramine. Subsequent increase in 5-HT in the P_2-fraction could contribute to the decreased rate in its disappearance from nerve-endings. There are two possible mechanisms for this: (1) the inhibition of uptake by imipramine increases the concentration of 5-HT at the receptor site and thus, by a feedback mechanism, decreases the rate of disappearance out of presynaptic neurons. (2) Imipramine decreases the release of 5-HT by interfering with the releasing mechanism at synaptic vesicles. The latter is supported by several findings (Zbinden, 1962; Brodie, Costa & others, 1968; Segawa, Kuruma & others, 1968; Segawa, 1970).

The inhibitory effect of desipramine and lofepramine on the uptake of noradrenaline intraventricularly into nerve-endings was demonstrated even at 90 min after noradrenaline while imipramine was ineffective in this respect (Fig. 4). The experiment has thus further demonstrated the *in vivo* selectivity of lofepramine for noradrenaline.

The S_2-fraction consists of microsomal particles and cytoplasm which are mainly derived from cell body and axon. Therefore, the fact that the antidepressants inhibited the increase of intraventricularly injected amines in the S_2-fraction suggests that the drugs can, to some extent, inhibit the amine uptake at both neuronal and axonal membranes.

Thus in spite of being a tertiary amine, lofepramine showed a qualitatively similar effect to desipramine in its preferential inhibition of noradrenaline uptake into noradrenergic neurons in the central nervous system.

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