Effect of antidepressant drugs on accumulation and disappearance of monoamines formed *in vivo* from labelled precursors in mouse brain

Tricyclic antidepressant drugs prevent the uptake of monoamines into noradrenaline and 5-hydroxytryptamine (5-HT) neurons (Dengler & Titus, 1961; Glowinski & Axelrod, 1964; Carlsson, Fuxe & others, 1966; Carlsson, Corrodi & others, 1969a, b), an effect which does not lead to changes in the levels of monoamines in tissues (Sulser, Watts & Brodie, 1962; Nybäck, Borzecki & Sedvall, 1968; Carlsson, & others, 1969a, b).

We have recently described the use of labelled tryptophan and tyrosine for the study of drug influences on monoamine metabolism in mouse brain (Schubert, Nybäck & Sedvall, 1970; Nybäck & Sedvall, 1970), methods that have the advantage that endogenous levels of brain amines are left unchanged. We now report the effect of some tricyclic antidepressants on the accumulation and disappearance of labelled 5-HT (^{3}H-5-HT), dopamine and noradrenaline formed in mouse brain *in vivo* after the administration of [^{3}H]tryptophan or [^{14}C]tyrosine intravenously.

5-HT *metabolism.* After an intravenous injection of [^{3}H]tryptophan to mice (male, NMRI, 18–22 g) the 5-HT store in brain is maximally labelled within 30 min (Schubert & others, 1970). Between 1–3 h after administration of the labelled precursor, ^{3}H-5-HT disappears from the brain at a rate which appears to be exponential and which is not increased by treatment with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (Schubert & others, 1970). Thus the disappearance of labelled 5-HT during the mentioned time interval is determined predominantly by the turnover rate of the amine.

Imipramine, desipramine, amitriptyline and nortriptyline (25 mg/kg) were administered intraperitoneally 1 h after the intravenous injection of [^{3}H]tryptophan (50 μCi/animal, 1·3 Ci/mmol). Groups of animals were killed 1 and 3 h after the precursor administration and the contents in brain of labelled tryptophan and 5-HT were determined as previously described (Schubert & others, 1970).

The dimethylated agents, amitriptyline and imipramine, retarded the rate of disappearance of ^{3}H-5-HT whereas the monomethylated derivatives had no significant ($P<0{\cdot}05$) effect (Table 1).

When the labelled precursor was administered by constant rate intravenous infusion for 20 min, the rate of ^{3}H-5-HT accumulation in brain was reduced by pretreatment of the animals with imipramine and chlorimipramine (Table 2).

Table 1. *Effect of some antidepressant drugs on the disappearance of* ^{3}H-5-HT *formed from* ^{3}H-*tryptophan in mouse brain.* Saline or drugs (25 mg/kg) were administered 1 h after i.v. injection of [^{3}H]tryptophan (50 μCi). Animals were killed 3 h after [^{3}H]trytophan administration. Figures represent mean value ± s.e. from 7 animals

Treatment	Time h	Total radioactivity counts/min × 10^3 g^{-1}	[^{3}H]Tryptophan counts/min × 10^3 g^{-1}	^{3}H-5-HT counts/min × 10^3 g^{-1}
Saline	1	84 ± 5·3	9·4 ± 0·5	1·72 ± 0·09
Saline	3	84 ± 5·8	3·4 ± 0·4	0·73 ± 0·04
Imipramine ..	3	75 ± 6·1	4·1 ± 0·5	0·99 ± 0·07*
Desipramine ..	3	72 ± 3·8	3·1 ± 0·3	0·85 ± 0·04
Amitriptyline ..	3	79 ± 6·0	3·5 ± 0·3	1·05 ± 0·08*
Nortriptyline ..	3	74 ± 2·9	2·6 ± 0·4	0·74 ± 0·06

* Differs from 3 h saline group ($P<0{\cdot}01$).

Desipramine, nortriptyline and amitriptyline did not significantly affect the ^{3}H-5-HT accumulation in brain. A reduction of the ^{3}H-5-HT accumulation by amitriptyline could, however, be masked by an increased precursor supply as indicated by the increased content of labelled tryptophan in the brain (Table 2).

Table 2. *Effect of some antidepressant drugs on the accumulation of* ^{3}H-5-HT *formed from* [^{3}H]*tryptophan in mouse brain.* [^{3}H]Tryptophan (40 μCi) was infused i.v. for 20 min starting 40 min after injection of saline or drugs (25 mg/kg). Animals were killed immediately after the infusion. Figures represent mean value ± s.e. from 6–8 animals

Treatment	Total radioactivity Counts/min × 10^3 g^{-1}	[^{3}H]Tryptophan Counts/min × 10^3 g^{-1}	^{3}H-5-HT Counts/min × 10^3 g^{-1}
Saline	113 ± 6·8	62 ± 2·5	1·39 ± 0·09
Imipramine	109 ± 7·0	69 ± 4·6	0·85 ± 0·05*
Desipramine ..	114 ± 4·0	63 ± 3·9	1·15 ± 0·07
Amitriptyline ..	138 ± 3·6	85 ± 4·7*	1·18 ± 0·08
Nortriptyline ..	117 ± 8·1	75 ± 6·1	1·21 ± 0·05
Chlorimipramine ..	115 ± 5·3	60 ± 5·9	0·77 ± 0·10*

* Differs from saline group ($P<0·001$).

Table 3. *Effect of some antidepressant drugs on the disappearance of catecholamines formed from* [^{14}C]*tyrosine in mouse brain.* Saline or drugs were administered 2 h (10 mg/kg) and 3, 4, 5 and 6 h (5 mg/kg) after injection of [^{14}C]tyrosine (10 μCi). Animals were killed 7 h after [^{14}C]tyrosine administration. Figures represent mean value ± s.e. from 4–6 animals

Treatment	Time h	Endogenous tyrosine μg/g	Tyrosine sp. activity counts/min μg^{-1}	[^{14}C]Dopamine counts/min g^{-1}	[^{14}C]Noradrenaline counts/min g^{-1}
—	2	9 ± 0·8	344 ± 28	757 ± 53	413 ± 17
Saline	7	10 ± 1·1	144 ± 24	216 ± 12	165 ± 20
Imipramine ..	7	9 ± 0·7	123 ± 21	227 ± 27	175 ± 12
Desipramine ..	7	9 ± 0·5	126 ± 13	217 ± 8	164 ± 12
Amitriptyline ..	7	9 ± 1·2	142 ± 7	265 ± 29	153 ± 13
Nortriptyline ..	7	8 ± 0·9	159 ± 25	250 ± 14	160 ± 10

Table 4. *Effect of some antidepressant drugs on the accumulation of catecholamines formed from* [^{14}C]*tyrosine in mouse brain.* [^{14}C]Tyrosine (7 μCi) was infused i.v. for 20 min starting 40 min after injection of saline or drugs (10 mg/kg). Animals were killed immediately after the infusion. Figures represent mean value ± s.e. from 4–6 animals

Treatment	Endogenous tyrosine μg/g	Tyrosine sp. activity counts/min × 10^3 μg^{-1}	[^{14}C]Dopamine counts/min g^{-1}	[^{14}C]Noradrenaline counts/min g^{-1}
Saline	14 ± 0·7	4·2 ± 0·29	980 ± 88	270 ± 20
Imipramine	12 ± 1·4	4·9 ± 0·62	1014 ± 88	237 ± 26
Desipramine	12 ± 1·1	5·1 ± 0·69	820 ± 100	160 ± 21†
Amitriptyline	11 ± 1·1	5·9 ± 0·32*	1060 ± 95	329 ± 24
Nortriptyline	12 ± 0·9	4·8 ± 0·51	873 ± 71	178 ± 16†

* Differs from saline group ($P<0·02$).
† Differs from saline group ($P<0·01$).

Catecholamine metabolism. After an intravenous injection of [^{14}C]tyrosine, labelled dopamine and noradrenaline accumulate in mouse brain during the first 30 min (Nybäck & others, 1968). Between 2 and 7 h after the precursor administration, the amines disappear from brain at a rate which seems to be exponential and which is not increased by synthesis inhibition with α-methyltyrosine (Nybäck & Sedvall, 1970). Thus the disappearance of labelled amines during the mentioned time interval is determined predominantly by the turnover rates of the amines.

The antidepressant drugs were administered intraperitoneally 2 h (10 mg/kg) and 3, 4, 5 and 6 h (5 mg/kg) after the intravenous injection of [^{14}C]tyrosine (10 μCi/animal, 355 mCi/mmol). Groups of animals were killed 2 and 7 h after the precursor administration and the contents in brain of endogenous tyrosine and labelled tyrosine, dopamine and noradrenaline were determined as previously described (Nybäck & Sedvall, 1968, 1970).

None of the antidepressants altered the rate of disappearance of labelled dopamine or noradrenaline from brain (Table 3).

When [^{14}C]tyrosine was administered by constant rate intravenous infusion for 20 min the accumulation of [^{14}C]noradrenaline was significantly reduced by pretreatment with desipramine and nortriptyline but not by their dimethylated derivatives (Table 4). None of the drugs caused any significant change in the accumulation of [^{14}C]dopamine. Amitriptyline increased the specific activity of tyrosine in brain ($P<0{\cdot}02$).

The present results demonstrate significant effects of dimethylated antidepressants on brain 5-HT metabolism but not on noradrenaline or dopamine metabolism. In contrast, the monomethylated derivatives had a significant effect on the metabolism of brain noradrenaline but not on that of dopamine or 5-HT. These findings are consistent with results obtained by other investigators using different methods (Carlsson & others, 1969a, b; Corrodi & Fuxe, 1969; Schildkraut, Schanberg & others, 1969; Ross & Renyi, 1969). The reduced amine accumulation and disappearance, found in the present study, indicate that dimethylated and monomethylated antidepressants decelerate synthesis and turnover of the transmitter in serotoninergic and noradrenergic neurons respectively. Such an effect could be mediated by the inhibition of amine re-uptake, if this leads to an increased receptor stimulation which by a negative feed-back mechanism inhibits nerve impulse activity in the presynaptic neuron.

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Inability of dextran to release kinin in rats

Ankier & Starr (1967) showed the hypotensive response to intravenous dextran in rats to be probably mediated by histamine and 5-hydroxytryptamine. Lecomte & Damas (1968) now suggest that plasma kinins may have an accessory role in this anaphylactoid reaction. I now report that dextran does not release kinin in rats.

Male Wistar albino rats, 150–175 g (Wellcome Laboratories, Beckenham, and Agricultural Research Council, Compton) were tested for reactivity to dextran on three occasions (250 mg/kg, i.p., *M* 110,000), and those which showed peripheral oedema (reactors) were separated from non-reactors. Reactor rats were anaesthetized with pentobarbitone (40 mg/kg, i.p.) and injected with heparin (50 units/kg, i.v.). Blood pressure was recorded from the right common carotid artery with a Condon mercury manometer, and drugs injected into the right femoral vein.

Dextran (250 mg/kg) elicited a fall in blood pressure in reactor rats which lasted 40–60 min, after an initial delay in onset of 2–5 min. A similar response was produced by ellagic acid (5 mg/kg), an activator of Hageman factor and plasma pre-kallikrein (Margolis, 1958; Ratnoff & Crum, 1964), but with a quicker onset (1–2 min) and a shorter duration (10–15 min). Tachyphylaxis developed to repeated injections of ellagic acid, indicating a progressive consumption of the substrate for plasma kallikrein in the blood (substrate 1—Jacobsen, 1966). However, the administration of dextran to kininogen-depleted rats still caused a typical fall in blood pressure. Pretreating rats with Soya Bean Trypsin Inhibitor (SBT1, 100 mg/kg) reduced the hypotensive activity of ellagic acid by about 64%, whereas this concentration did not affect the action of dextran (Table 1). From these observations it seems unlikely that dextran activates plasma kinin-forming enzymes to a significant extent, since inhibition of plasma kallikrein with SBT1, or reducing the level of its substrate in the blood, did not modify the course of the reaction to dextran. On the other hand, both of these procedures attenuated the hypotensive action of ellagic acid, a compound which is known to activate plasma kallikrein. Another possibility also investigated was that dextran in some way liberated glandular

Table 1. *Effects on the blood pressure responses to dextran in reactor rats of soya bean trypsin inhibitor (SBT1, 100 mg/kg, i.v.) and ellagic acid, and of Trasylol (100 000 units/kg, i.v.) and pancreatic kallikrein*

Drug	dose (mg/kg)	Inhibitor	% fall in blood pressure (± s.e.) Untreated	After inhibition
Dextran	250	SBTI	58·8 ± 6·9	54·3 ± 2·3
Ellagic acid	5	SBTI	49·5 ± 4·8	17·8 ± 1·2*
Dextran	250	Trasylol	65·1 ± 9·2	58·2 ± 5·0
Pancreatic kallikrein	150	Trasylol	27·4 ± 2·4	13·4 ± 2·8*

* Inhibition = 64% ($P < 0.001$).
* Inhibition = 51% ($P < 0.005$).