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Amitriptyline and Clomipramine Increase the Concentration of Administered L-Tryptophan in the Rat Brain

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Abstract

The tricyclic antidepressant amitriptyline has been shown to reduce concentrations of large neutral amino acids (LNAA) in rat plasma. Compounds with that property might interact with such amino acids used as therapeutic agents with a central site of action by causing a change in the relationship between the administered LNAA and its endogenous LNAA competitors for carrier-mediated transport through the blood–brain barrier into the brain. This study was performed to investigate if the antidepressant agents amitriptyline and clomipramine could, by such a mechanism, increase brain concentrations of administered tryptophan.

Intraperitoneal administration of L-tryptophan alone ($100\,mg\,kg^{-1}$) resulted in an increase in the concentration of tryptophan in the rat brain from $14 \pm 0{\cdot}7$ to $100 \pm 4{\cdot}3\,nmol\,g^{-1}$ compared with rats given saline only. When rats were given tryptophan with amitriptyline ($25\,mg\,kg^{-1}$, i.p.) or clomipramine ($25\,mg\,kg^{-1}$, i.p.) brain concentrations of tryptophan were increased even further, to $150 \pm 4{\cdot}5$ and $157 \pm 10{\cdot}2\,nmol\,g^{-1}$, respectively. Administration of L-tryptophan alone resulted in an increase in the rat plasma tryptophan ratio [(concentration of tryptophan)/(total concentration of LNAAs)] from $0{\cdot}14 \pm 0{\cdot}003$ to $0{\cdot}42 \pm 0{\cdot}011$ compared with rats given saline only. When rats were given tryptophan with amitriptyline or clomipramine the plasma tryptophan ratios were increased even further to $0{\cdot}52 \pm 0{\cdot}017$ and $0{\cdot}54 \pm 0{\cdot}025$, respectively. All these effects were statistically significant ($P < 0{\cdot}001$).

These findings support the hypothesis that tricyclic antidepressants could interact with administered tryptophan by changing the relationship in plasma between tryptophan and its endogenous LNAA competitors for transport into the brain, resulting in higher concentrations of tryptophan in the brain. It is possible that this could be the mechanism of the previously reported finding that clomipramine and tryptophan potentiate each other in the treatment of depression.

To influence the synthesis and action of monoaminergic neurotransmitters in the brain several large neutral amino acids (LNAA) have been used as therapeutic agents, e.g. α-methyldopa in hypertension and L-dopa in Parkinson's disease. The amino acid L-tryptophan, precursor to the monoaminergic neurotransmitter 5-hydroxytryptamine (5-HT), has also been used for treatment of depression (Coppen 1967). It has been suggested that this treatment increases the availability of tryptophan in the brain and consequently the synthesis of 5-HT (Wurtman & Fernstrom 1976; Fernstrom 1983; Milner & Wurtman 1986), although it has not been possible to demonstrate a clear antidepressant effect of tryptophan when used in monotherapy (Murphy et al 1974; Mendels et al 1975; Chouinard et al 1979). Other groups have reported that L-tryptophan given with a tricyclic antidepressant such as clomipramine (Wålinder et al 1976) or amitriptyline (Thomson et al 1982), potentiates the effect of the tricyclic antidepressant. The pharmacological basis of the potentiation is yet to be explained.

To reach its central site of action in the brain, tryptophan must be transported from the plasma through the blood–brain barrier. This transport is carrier-mediated and occurs in competition with

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that of other LNAAs (tyrosine, valine, phenylalanine, isoleucine and leucine) (Oldendorf 1975; Pardridge 1977), thus the relationship between the concentration of tryptophan and the total concentration of its endogenous LNAA competitors in plasma is crucial to the transport of tryptophan into the brain. Compounds capable of influencing the concentrations of endogenous LNAAs in plasma might thus interact with administered LNAAs with a central site of action. We have previously demonstrated such interactions between isoprenaline (Eriksson & Carlsson 1982) and ethanol (Eriksson et al 1979), both of which reduce concentrations of LNAAs in rat plasma, and administered L-dopa and L-tryptophan. When given with isoprenaline or ethanol, concentrations of these amino acids in the rat brain reach higher levels than when rats are given these amino acids alone.

Many tricyclic antidepressants have been shown to affect the concentrations of the monoamine precursor amino acids tyrosine and tryptophan in the plasma and brain of the rat (Tagliamonte et al 1971; Kim et al 1982; Redfern & Martin 1985; Edwards et al 1988; Edwards & Sorisio 1988) and in the plasma in man (Thomas et al 1987; Fekkes et al 1997). These reports do not, however, give any data on the effects of tricyclic antidepressants on the plasma concentrations of those LNAAs with which tyrosine and tryptophan compete for carrier-mediated transport into the brain.

We have previously observed that a single dose of amitriptyline reduces concentrations of all LNAAs in rat plasma (Eriksson & Eriksson 1995). Thus we expected that amitriptyline and possibly other tricyclic antidepressants might interact with administered tryptophan by influencing the relationship between tryptophan and its endogenous LNAA competitors in plasma, and thus the transport of tryptophan from plasma into the brain. This hypothesis has been tested in this study.

Materials and Methods

Animals

Experiments were performed on six groups of seven male Sprague-Dawley rats, 180–220 g, previously housed for more than one week in a room maintained on a 14–10 h light–dark cycle with the lights switched on at 0500 h. Before the experiment, which was approved by the ethical committee for animal experiments in Göteborg, all rats had free access to food (R34-EWOS-ALAB (Sweden)-Grower-maintenance feed, 16·5% crude protein) and water.

Experimental design

The experiment began at 0900 h. Each animal was injected intraperitoneally 60 and 40 min before death. In the first injection the rats were given saline, amitriptyline (25 mg kg^{-1}) or clomipramine (25 mg kg^{-1}). In the second injection tryptophan (100 mg kg^{-1}) or saline was administered.

The rats were killed by decapitation. Immediately after death, blood (approx. 5 mL) was collected directly from the neck into a tube containing EDTA solution (1%, 0·5 mL) and immediately centrifuged. The brains were removed and frozen on dry ice. The brains and plasma samples were stored at −70°C until analysis.

The concentrations of the LNAAs in plasma and whole brain were determined according to procedures described elsewhere (Lindroth & Mopper 1979; Eriksson & Carlsson 1988).

Data analysis

For clarity the total concentrations of all LNAAs except tryptophan are presented as one variable (calculated as the sum of the molar concentrations in plasma or brain of the amino acids tyrosine, valine, phenylalanine, isoleucine and leucine).

Comparisons between groups were made to elucidate the effects of amitriptyline and clomipramine, both alone and in combination with tryptophan, on the absolute concentrations of tryptophan and the total concentration of its LNAA competitors in plasma and brain and on the relative concentration of tryptophan in plasma (calculated as the ratio [Trp]/[ΣLNAA]).

The statistical significance of differences was assessed by one-way analysis of variance. To elucidate statistically significant differences between groups, post-hoc assessments were performed by the Tukey HSD method.

For assessment of differences between groups of plasma and brain tryptophan concentrations statistical analysis was performed separately for those groups to which tryptophan had been administered and for those to which it had not.

Results

Effects on the concentrations of tryptophan and its LNAA competitors in plasma (Table 1)

Administration of L-tryptophan (100 mg kg^{-1}) did not affect the total plasma concentration of other LNAAs but elicited an increase of over 300% in the plasma concentration of tryptophan. The relative concentration of tryptophan was thus increased from 14% to over 40% of the total plasma LNAA concentration in all groups given tryptophan.

Amitriptyline alone caused a decrease both in the concentration of tryptophan and in the total

Table 1. A. Effects of amitriptyline, clomipramine, tryptophan and B. combinations of amitriptyline and clomipramine with tryptophan on the concentration of tryptophan and the total concentration of large neutral amino acids (LNAA) except tryptophan in rat plasma and brain.

A.

Variable	Compounds administered			
	Saline† Saline‡ (n = 7)	Amitriptyline†§ Saline‡ (n = 7)	Clomipramine†§ Saline‡ (n = 7)	Saline† Tryptophan‡¶ (n = 7)
Plasma tryptophan (nmol mL^{-1})	100 ± 4·0	64 ± 6·6***‡‡‡	90 ± 6·2	424 ± 21·8
Plasma tryptophan (ratio)††	0·14 ± 0·003	0·12 ± 0·006*‡‡‡	0·13 ± 0·004	0·42 ± 0·011
Brain tryptophan (nmol g^{-1})	14 ± 0·7	14 ± 0·8	14 ± 0·9	100 ± 4·3
Plasma ΣLNAA‡‡ except tryptophan (nmol mL^{-1})	608 ± 26·9	463 ± 32·6*‡‡‡	600 ± 29·0	590 ± 38·8
Brain ΣLNAA‡‡ except tryptophan (nmol g^{-1})	342 ± 7·8	360 ± 13·8	378 ± 13·9	300 ± 16·0

B.

Variable	Compounds administered		Statistics§§
	Amitriptyline†§ Tryptophan‡¶ (n = 7)	Clomipramine†§ Tryptophan‡¶ (n = 5)	
Plasma tryptophan (nmol mL^{-1})	457 ± 17·1	557 ± 27·4**§§§	$F = 10·0$; $P < 0·001$¶¶
			$F = 9·2$; $P < 0·005$†††
Plasma tryptophan (ratio)††	0·52 ± 0·017***§§§	0·54 ± 0·025***§§§	$F = 4·3$; $P < 0·05$¶¶
			$F = 15·2$; $P < 0·001$†††
Brain tryptophan (nmol g^{-1})	150 ± 4·5***§§§	157 ± 10·2***§§§	$F = 0·1$; NS¶¶
			$F = 27·5$; $P < 0·001$†††
Plasma ΣLNAA‡‡ except tryptophan (nmol mL^{-1})	418 ± 24·8***‡‡‡	469 ± 27·1	$F = 7·6$; $P < 0·001$
Brain ΣLNAA‡‡ except tryptophan (nmol g^{-1})	295 ± 17·4	260 ± 9·8**‡‡‡	$F = 9·5$; $P < 0·001$

Results are given as means ± s.e.m. † Injection given 60 min before death; ‡ injection given 40 min before death; § 25 mg kg^{-1} intraperitoneally; ¶ 100 mg kg^{-1} intraperitoneally; †† calculated as the molar concentration of tryptophan divided by [ΣLNAA]; ‡‡ ΣLNAA is calculated as the sum of the molar concentrations in plasma or brain of the amino acids tyrosine, tryptophan, valine, phenylalanine, isoleucine and leucine; §§ one-way analysis of variance followed by Tukey HSD test to assess statistically significant differences in selected comparisons (shown as asterisks); ¶¶ calculated separately for those groups which were not given tryptophan; ††† calculated separately for those groups which were given tryptophan. * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ significantly different from: ‡‡‡ results from the saline–saline group; §§§ results from the saline–tryptophan group.

concentration of the other LNAAs in plasma. The relative concentration of tryptophan was also reduced. Administration of a combination of amitriptyline and tryptophan resulted in a decrease in the total plasma concentration of the other LNAAs. This combination did not, however, affect the concentration of tryptophan in plasma compared with tryptophan-injected controls. Consequently the combination of amitriptyline and tryptophan caused a statistically significant increase in the relative concentration of tryptophan in plasma, compared with animals given tryptophan only.

In contrast with amitriptyline, clomipramine alone did not influence the absolute plasma concentration of tryptophan or the total concentration of the other LNAAs. Administered with tryptophan, however, clomipramine elicited a statistically significant reduction in the plasma concentrations of the LNAAs tyrosine, phenylalanine and leucine (data not shown) compared with rats given saline only and with rats given either clomipramine or tryptophan. The reduction in the total concentration of LNAAs did not, however, reach statistical significance ($P = 0·53$).

Clomipramine administered with tryptophan elicited a statistically significant 32% increase in the plasma concentration of tryptophan compared with animals given L-tryptophan only, thus increasing the relative concentration of tryptophan.

Effects on the concentrations of tryptophan and other LNAAs in the brain (Table 1)

L-Tryptophan alone did not significantly influence the total brain concentration of the other LNAAs.

Compared with saline-injected controls the brain concentration of tryptophan was, however, increased by nearly 600% after administration of tryptophan.

Neither amitriptyline nor clomipramine, given separately, influenced the brain concentrations of tryptophan or the total brain concentration of the other LNAAs compared with saline-injected controls. Administration of amitriptyline and clomipramine with L-tryptophan elicited a statistically significant increase of more than 50% in the brain concentration of tryptophan compared with rats given L-tryptophan only.

L-Tryptophan in combination with clomipramine resulted in a decrease in total brain LNAA concentration (tryptophan excluded) compared with animals not given tryptophan. L-Tryptophan alone or in combination with amitriptyline had no statistically significant effect on total brain LNAA concentration.

Discussion

L-Tryptophan has previously been shown to potentiate the effects of tricyclic antidepressants (Wålinder et al 1976; Thomson et al 1982). To the best of our knowledge there has been no pharmacological explanation of this interaction.

These results support our hypothesis that concomitant administration of a tricyclic antidepressant and L-tryptophan gives rise to a higher concentration of tryptophan in the brain than administration of L-tryptophan alone. The data also support the contention that this increased concentration of tryptophan is the result of a tricyclic antidepressant-induced change in the relationship between tryptophan and its endogenous competitors in favour of tryptophan. These findings offer an explanation of the clinical finding of potentiation of the action of tricyclic antidepressants by tryptophan in depressive illness.

Only amitriptyline caused a reduction in concentrations of the endogenous LNAAs in plasma when given alone. Although clomipramine alone had no such effect, when administered with tryptophan, which by itself did not affect the concentrations of the other LNAAs, clomipramine exerted an LNAA-reducing effect which reached statistical significance for the amino acids tyrosine, phenylalanine and leucine (data not shown).

Clearly amitriptyline and clomipramine do not influence plasma LNAA concentrations via a common mechanism. The effects on plasma LNAA concentrations of amitriptyline alone and clomipramine given with tryptophan were, however, similar. Both led to reduced plasma concentrations of at least some of those endogenous LNAAs with which tryptophan competes for transport into the brain and, as a consequence, increased the relative concentrations of tryptophan in plasma.

Another difference between amitriptyline and clomipramine, when these compounds were administered with tryptophan, was that the combination of clomipramine and tryptophan caused not only a reduction in the concentration of several endogenous LNAAs in plasma, but also an increase in the absolute plasma concentration of tryptophan, compared with animals given tryptophan alone. This increase in the absolute concentration of tryptophan in plasma obviously added to the increase in the relative concentration of this amino acid. No such effect was seen after the combination of amitriptyline and L-tryptophan. Why clomipramine, but not amitriptyline, in combination with L-tryptophan elicits an increase in plasma tryptophan is not fully understood. It is possible that inhibition of liver tryptophan pyrrolase activity is involved. This assumption is in line with a report that several antidepressants cause such inhibition (Badawy & Evans 1982).

How amitriptyline and a combination of clomipramine and L-tryptophan reduce plasma LNAAs is not clear. Edwards & Sorisio (1988) have suggested that imipramine reduces the concentration of tyrosine in rat plasma by stimulating β-adrenergic receptors. They based their suggestion on the finding that administration of the β-adrenoceptor antagonist propranolol partly inhibited the imipramine-induced reduction in plasma tyrosine. However, in the same experiment they found no similar inhibition by propranolol of the imipramine-induced reduction in plasma tryptophan. Thus other mechanisms might be operating. The mechanism behind the plasma LNAA-reducing effect of the combination of clomipramine with tryptophan is another area which must be further explored.

It is also necessary to investigate if, and to what extent, a combination of amitriptyline or clomipramine and L-tryptophan is capable of increasing brain 5-HT activity. Such experiments should be performed with doses of L-tryptophan which do not by themselves give rise to brain tryptophan concentrations above the level at which tryptophan hydroxylase, the enzyme catalysing the rate-limiting step in 5-HT synthesis (Carlsson & Lindqvist 1972), is saturated.

The suggested mechanism behind the interaction between tricyclic antidepressants and L-tryptophan in the treatment of depression might prove useful in the development of this combined treatment. From a clinical standpoint it is, of course, necessary to investigate if therapeutic doses of amitriptyline and

clomipramine given with L-tryptophan have similar effects on the plasma LNAA pattern in man as found in rat.

The importance of plasma amino acid determinations in the biochemical diagnosis and treatment of depression has been emphasized by Møller and coworkers who, in a series of studies, have been able to demonstrate that the ratio of both tyrosine and tryptophan to their competing LNAAs is of predictive value in the choice of treatment for depression (Møller 1985; Møller et al 1985).

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