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Effects of Amitriptyline on Rat Plasma and Brain Content of Monoamine Precursors and Other Large Neutral Amino Acids

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Abstract

Amitriptyline caused decreased concentrations in rat plasma of the amino acids tyrosine and tryptophan, which are precursors to monoaminergic neurotransmitters, and also of those other large neutral amino acids (LNAAs) (valine, phenylalanine, leucine and isoleucine) with which tyrosine and tryptophan compete for the carrier-mediated transport from plasma into the brain.

The various LNAAs were, however, not decreased to the same extent. Thus, the relative concentrations, calculated as the ratio of each one of them to the total concentration of LNAAs, were also influenced by amitriptyline. The relative concentrations of tryptophan were decreased while the relative concentrations of phenylalanine and leucine were increased. Those of tyrosine, valine and isoleucine were not influenced by amitriptyline. The relative concentrations of the LNAAs in plasma are considered to be of importance to how much of each of them that will be transported into the brain. Based on this assumption one would have expected that the amitriptyline-induced changes in the relationships between different LNAAs would have caused changes in their brain concentrations in accordance with their relative concentrations in plasma. However, amitriptyline caused decreased concentrations of all LNAAs in rat whole brain.

This finding cannot be explained by altered relationships between the LNAAs but must be explained by other mechanisms. Nevertheless, this effect of amitriptyline on brain LNAA concentrations might be of importance to monoamine function.

The effects of tricyclic antidepressant agents on plasma concentrations of the monoamine precursors tyrosine and tryptophan have been investigated in several animal studies (Tagliamonte et al 1971; Kim et al 1982; Redfern & Martin 1985; Edwards & Sorisio 1988; Edwards et al 1988). Such experiments have been undertaken to elucidate possible mechanisms, additional to the monoamine reuptake inhibition, by which these compounds could exert their antidepressant action. The main hypothesis has been that antidepressant agents, by altering the plasma concentration of tyrosine or tryptophan, could influence the brain content of these amino acids and consequently the synthesis of biogenic amines in the brain.

In an early investigation on rat, Tagliamonte et al (1971) showed that an acute dose of desipramine (10 mg kg^{-1}) after 90 min caused decreased plasma concentrations of both tyrosine and tryptophan. Brain tyrosine concentration was found to be reduced while brain tryptophan was unchanged. Kim et al (1982) found an increase in the serum free tryptophan concentrations but no alteration in the total serum tryptophan in rat after chronic administration of amitriptyline (14 days, 10 mg kg^{-1}, i.p.). Brain amino acid content was not investigated. Redfern & Martin (1985) also found increased concentrations of free tryptophan after chronic administration of zimelidine and clomipramine, but found decreased concentrations of free tryptophan after chronic administration of imipramine in rat experiments (all drugs administered 14 days in drinking water, 200 $\mu g\,mL^{-1}$). No data on brain amino acids were given. In an investigation of eleven different antidepressant drugs, acutely administered to rat, Edwards et al (1988) found that almost all of them, 90 min after administration, caused decreased concentrations of tyrosine in both plasma and brain. The same group (Edwards & Sorisio 1988) has also reported that an acute dose of imipramine (90 min, 20 mg kg^{-1}) caused a decrease in rat plasma concentrations of both tyrosine and tryptophan. In the same experiment they found a decrease in brain tyrosine and an increase in brain tryptophan. Furthermore, Thomas et al (1987) reported lower concentrations of tryptophan in plasma in unipolar depressed patients on treatment with tricyclic antidepressants compared with patients without such medication.

The above cited reports support the contention that tricyclic antidepressants are capable of altering plasma concentrations of tyrosine and tryptophan. However, since these amino acids are transported from plasma into the brain by a saturable carrier in competition with other large neutral amino acids (LNAA) (tyrosine, tryptophan, valine, phenylalanine, isoleucine and leucine) (Oldendorf 1975; Pardridge 1977), such findings alone might not be enough to predict and explain altered amino acid concentrations in the brain. Notwithstanding, changes in the relationships between the concentrations of LNAAs in plasma could be expected to influence concentrations of the various LNAAs in brain (Wurtman & Fernstrom 1976; Fernstrom 1983; Milner & Wurtman 1986).

To investigate the hypothesis that antidepressant agents

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could alter plasma amino acid concentrations and the relationships between these concentrations in a way which could be expected to influence monoamine precursor amino acid concentrations in the brain, we have investigated the acute effects of amitriptyline on all LNAAs in rat plasma and brain.

Materials and Methods

Animals

Male Sprague-Dawley rats, 300 g, were purchased from ALAB, Sweden. Before use the animals were housed for at least one week in a room maintained on a 14/10 h light/dark cycle. The lights were switched on at 0500 h and switched off at 1900 h. During the weeks before the experiments the animals had free access to food (R34-EWOS-ALAB (Sweden), Grower maintenance feed, 16·5% crude protein) and water. The experiments took place at 0900 h.

Experimental design

In one experiment, groups of rats (n = 6) were injected intraperitoneally with amitriptyline HCl (Sigma) in doses of 3·2, 6·25, 12·5, 25 or 50 mg kg^{-1}. The animals received a constant volume of 10 mL kg^{-1}. Control rats were given the same volume of saline. Sixty minutes after the respective injection the animals were killed.

In a second experiment, three groups of rats (n = 6) were given amitriptyline HCl (25 mg kg^{-1}; 10 mL kg^{-1}) intraperitoneally. Three control groups received saline in equivalent volumes. The animals were killed 30, 120 or 240 min after the injection.

Determination of large neutral amino acids (LNAA)

In both experiments the animals were killed by decapitation and about 5 mL blood collected into a tube containing 0·5 mL 1% EDTA solution. Immediately after death the brains were taken out and frozen on dry ice. The concentrations of LNAAs were determined in plasma and brains according to the following procedures modified from Lindroth & Mopper (1979).

Plasma (1 mL) was deproteinized with 0·5 mL 4 M $HClO_4$, diluted with 3·5 mL distilled water, and mixed with 75 μL 10% EDTA and 75 μL 5% $Na_2S_2O_5$. The plasma samples were centrifuged at 10 000 *g* for 10 min and filtered before assay.

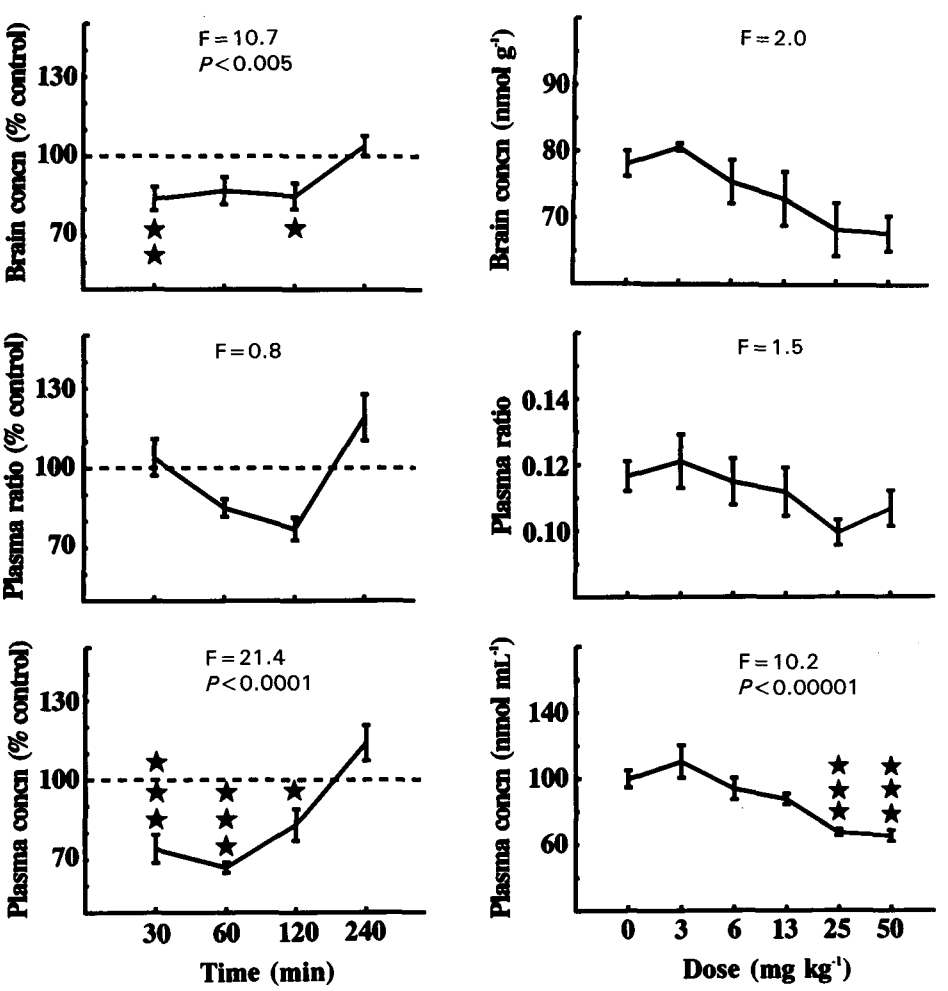

FIG. 1. Effects of amitriptyline on rat brain and plasma concentrations of tyrosine. Left panels, time course after intraperitoneal injection of 25 mg kg^{-1} amitriptyline. Right panels, effect of dose of amitriptyline at 60 min. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with controls.

Brains were homogenized in a mixture containing 10 mL 0·4 M $HClO_4$, 150 μL 10% EDTA and 150 μL 5% $Na_2S_2O_5$. The homogenates were centrifuged in the same way as were the plasma samples.

In both brain and plasma samples the amino acids valine, phenylalanine, leucine and isoleucine were assayed in a gradient HPLC system consisting of two HPLC pumps (LKB model 2150) and an HPLC controller (LKB model 2152). Samples were loaded with a high-pressure sampling valve (Rheodyne model 7125) with a 20 μL sample loop. The column (200 × 4·6 mm) was packed with Nucleosil (RP-18·5 μm). As an internal standard *p*-fluoro-DL-phenylalanine (Sigma) (100 μM) was used. A solution containing 20 μM of each of the amino acids to be detected was used as external standard. The amino acids in the samples and in the external standards were derivatized with *o*-phthaldialdehyde (Merck). The reagent contained a mixture of 135 mg *o*-phthaldialdehyde in 2·5 mL methanol, 22·5 mL 0·4 M borate buffer (pH 9·5) and 100 μL 2-mercaptoethanol. Two hundred microlitres of this mixture and 10 μL internal standard solution were added to 30 μL sample and external standard, respectively, 2 min before loading. The mobile phase was a mixture of 0·05 M sodium citrate buffer and 0·05 M phosphate buffer in a proportion of four to one (pH 6·5) and methanol in a gradient from 40 to 80% for 20 min. The flow rate was 1 mL min^{-1}. The fluorescence was detected with a Fluoro Monitor III (LDC model 1311).

Since the amino acids tyrosine and tryptophan were not completely separated from β-alanine and cystathionine, respectively, these amino acids were analysed in an isocratic chromatographic system with electrochemical detection. Each of these systems consisted of a Minipump (LDC model 395), an injector (Rheodyne, model 7125) with a 100-μL-sample loop, a Nucleosil (RP-18·5 μm) column (150 × 4·6 mm) and an electrochemical detector (BAS model LC-3A). The detector potential was 0·95 V.

In the tyrosine analysis, 100 μL tyramine solution (73 μM), used as internal standard, was added to 1 mL of each sample and external standard. A solution containing 5·5 μM tyrosine was used as external standard. To avoid interference from catechols, these were isolated before analysis in the following way. Aluminium oxide (20 mg) and 0·5 mL 3 M Tris buffer, pH 8·6, was added to 1 mL of each sample and external standard. The tubes were shaken for 15 min, and 0·5 mL of the supernatant was transferred to another tube containing 100 μL 4 M $HClO_4$. The mobile

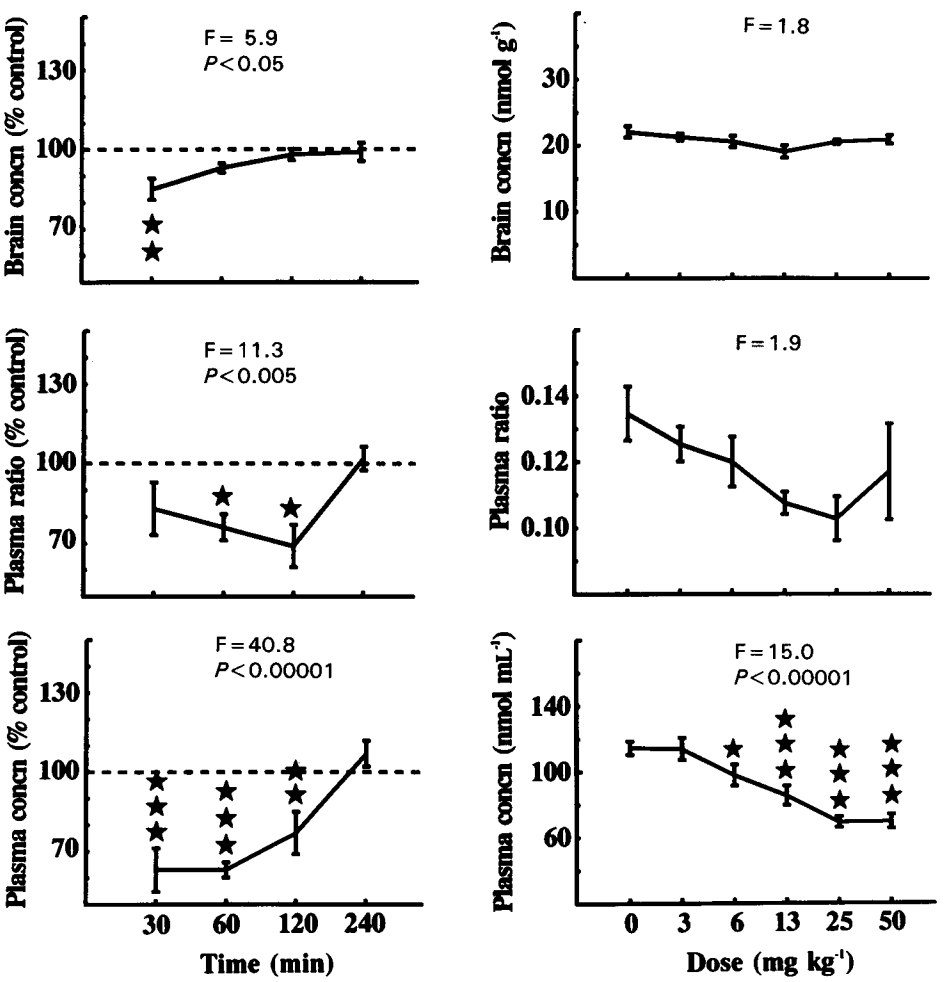

FIG. 2. Effects of amitriptyline on rat brain and plasma concentrations of tryptophan. Left panels, time course after intraperitoneal injection of 25 mg kg^{-1} amitriptyline. Right panels, effect of dose of amitriptyline at 60 min. *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ compared with controls.

phase in the tyrosine analysis was a citrate phosphate buffer (0·026 M K_2HPO_4, 0·031 M citric acid, 10% methanol, octylhydrogensulphate (Merck) 50 mg L^{-1} and EDTA (Merck) 20 mg L^{-1}). The buffer pH was 2·7. The flow rate 2 mL min^{-1}.

In the tryptophan analysis, DL-3-indol lactic acid (Sigma) was used as internal standard at a concentration of 5 μM; 100 μL was added to 0·5 mL samples and external standards, respectively. The tryptophan concentration in the external standard was 0·25 μM. The mobile phase was a citrate buffer (0·026 M sodium citrate, 0·031 M citric acid, 10% methanol, octylhydrogensulphate (Merck) 50 mg L^{-1} and EDTA (Merck) 20 mg L^{-1}. The buffer pH was 4·0. The flow rate was 2 mL min^{-1}.

Analysis of data

For each plasma sample the relative concentrations of LNAAs, expressed as the ratio of each LNAA to the total concentration of LNAAs in that sample, was calculated. The total concentration of LNAAs was also calculated in each plasma sample. Statistical significances were assessed by an one-way analysis of variance followed by a *t*-test in which each dose was compared with saline-injected controls. In the experiment in which the effects of amitriptyline on plasma and brain amino acids at various time intervals after administration of amitriptyline were investigated, the statistical significances were assessed by a two-way analysis of variance with treatment (amitriptyline or saline) and time (min) as sources of variance. When the analysis of variance had demonstrated a statistically significant variance with treatment, *t*-tests were used to assess statistically significant differences between groups given amitriptyline and control groups.

The data obtained from the group of rats which was given amitriptyline at a dose of 25 mg kg^{-1} in the experiment in which various doses of amitriptyline were administered 60 min before death, were included also in the statistical analysis of the data obtained in the experiment in which the effects on amino acids at various time intervals after the administration of amitriptyline were investigated. These data are included both in the time curves and in the dose curves in Figs 1–7.

Before the assessments of statistical significances all data were assessed by Dixon's gap test (Dixon 1951) to validate rejection of outliers. By this procedure data obtained from two of 65 plasma samples and three of 65 brain samples were excluded from the statistical analysis.

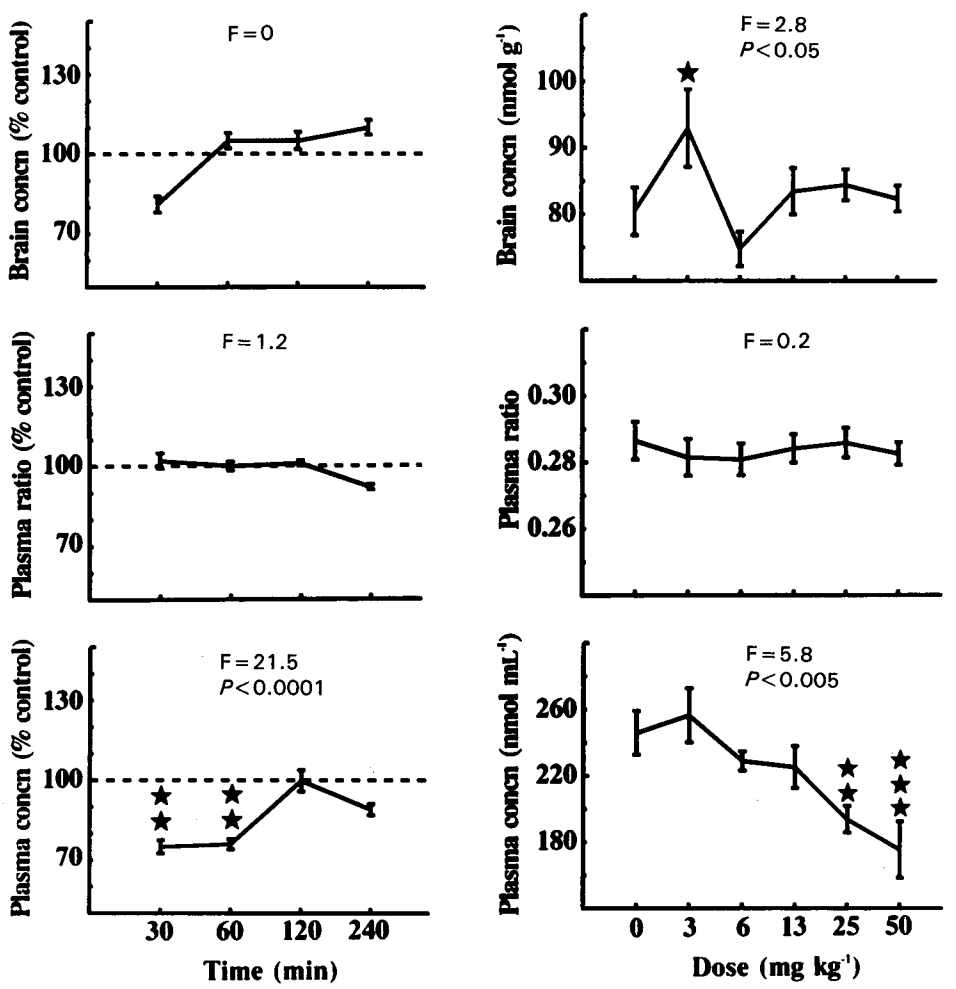

FIG. 3. Effects of amitriptyline on rat brain and plasma concentrations of valine. Left panels, time course after intraperitoneal injection of 25 mg kg^{-1} amitriptyline. Right panels, effect of dose of amitriptyline at 60 min. $*P < 0·05$, $**P < 0·01$, $***P < 0·001$ compared with controls.

Results and Discussion

The results of all analyses are summarized in Figs 1–8.

Amitriptyline caused decreased concentrations of tyrosine and tryptophan in plasma compared with controls and also decreased concentrations of those other LNAAs in plasma with which tyrosine and tryptophan have to compete for the carrier mediated transport into the brain. The decreases in the concentration of those other LNAAs were, however, less pronounced than those demonstrated for tyrosine and tryptophan. Therefore, the relative concentrations of these two amino acids were decreased, while the relative concentrations of the others were increased (phenylalanine, and leucine) or unchanged (valine and isoleucine).

The relative concentrations of the LNAAs are considered to be of importance to how much of each of the LNAAs that will be transported into the brain via the specific LNAA carrier, for which these amino acids compete. Based on this assumption one would have expected that the amitriptyline-induced changes in the relationships between different LNAAs would have caused changes in brain concentrations of these amino acids in the same directions as were the relative concentrations in plasma changed. In agreement with this contention and also in agreement with previous findings by Tagliamonte et al (1971) and by Edwards et al (1988), we found that amitriptyline caused decreased concentrations of tyrosine in rat whole brain. However, amitriptyline caused decreased concentrations not only in the concentrations of tyrosine but of all LNAAs in brain. Even the brain concentration of phenylalanine, the relative concentration of which was increased in plasma after the administration of amitriptyline, was decreased in brain after amitriptyline administration. Only for the amino acids tyrosine and tryptophan, were the variations in plasma concentrations, caused by various doses of amitriptyline, positively correlated to the brain concentration of the respective amino acid. Furthermore, the relative plasma concentrations were not significantly better correlated to brain concentrations than were the absolute plasma concentrations.

These findings cannot be explained by altered relationships between various LNAAs in plasma. The demonstrated effects of amitriptyline on brain LNAA content must, at least partly, be mediated via some other mechanism. The contention that the regulation of brain LNAA content is regulated by the relationships between various LNAAs in plasma is obviously

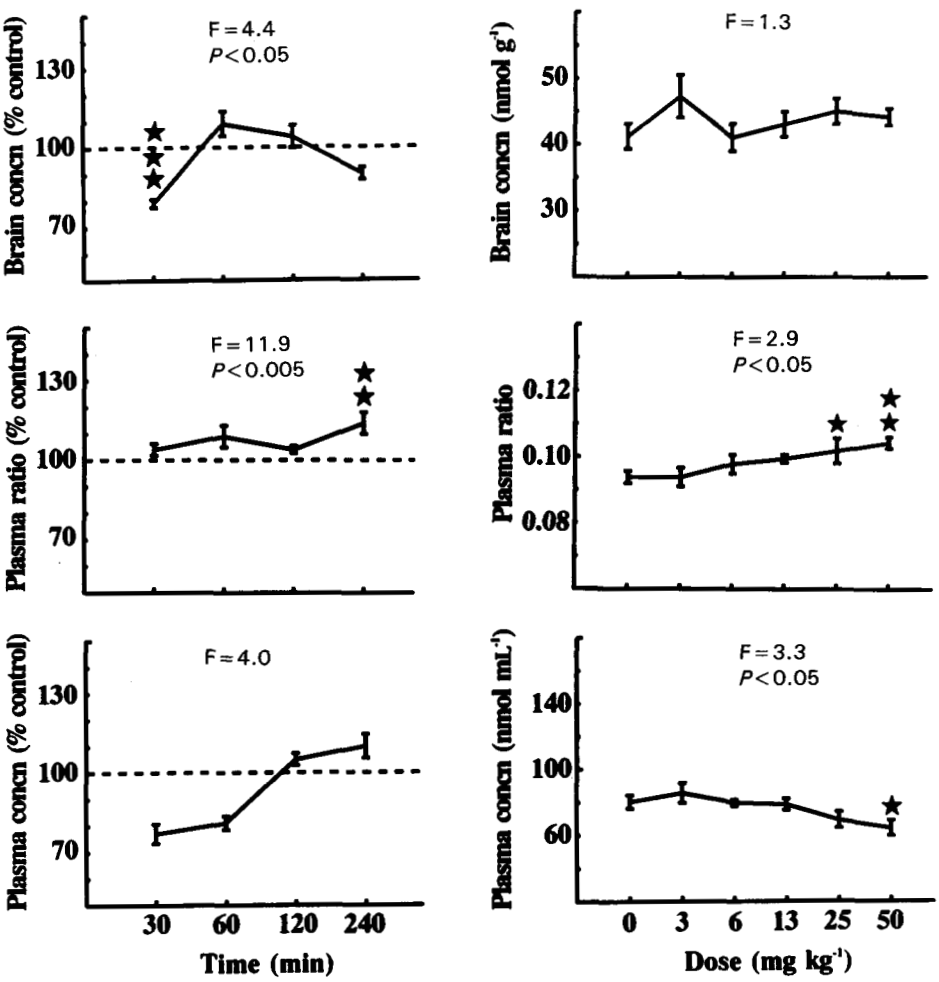

FIG. 4. Effects of amitriptyline on rat brain and plasma concentrations of phenylalanine. Left panels, time course after intraperitoneal injection of 25 mg kg^{-1} amitriptyline. Right panels, effect of dose of amitriptyline at 60 min. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with controls.

not generally valid; in a previous report (Voog & Eriksson 1992) we have demonstrated that those diurnal variations, seen in the relative concentrations of LNAAs in plasma, are not correlated to variations in brain concentrations of those amino acids.

How amitriptyline exerts its LNAA decreasing effect in plasma and brain is not clear. Edwards & Sorisio (1988) have suggested that the decreased concentrations of tyrosine seen in rat plasma after imipramine administration could be mediated via β-adrenoceptor stimulation. A similar explanation might be considered also for amitriptyline. This suggestion is supported by the present finding that amitriptyline caused decreased concentrations of all LNAAs in plasma. We have previously reported (Eriksson et al 1984) that the β-adrenoceptor agonist isoprenaline causes a similar decrease in all LNAAs in rat plasma. On the other hand isoprenaline causes, in contrast to amitriptyline, increased relative concentrations of tyrosine and tryptophan in plasma and also increased concentrations of both these amino acids in rat whole brain (Eriksson et al 1984). In fact, isoprenaline causes, also in contrast to amitriptyline, increased concentration of all LNAAs taken together in rat brain (Eriksson & Carlsson 1988).

Even if the amitriptyline-induced effect on plasma LNAA concentrations is mediated via a β-adrenergic mechanism, it is difficult to explain the amitriptyline-induced decrease in rat brain LNAAs by a possible direct or indirect stimulation of β-adrenoceptors. Other mechanisms, beside the proposed β-adrenergic stimulation, must be considered in this context.

Another side of the demonstrated amitriptyline-induced decrease in the total plasma LNAA concentration is that such a decrease could be expected to cause an increased transport of administered LNAAs (e.g. tryptophan) from plasma into the brain, if administered together with amitriptyline. We have previously demonstrated such an effect on brain concentrations of administered amino acids given with ethanol (Eriksson & Carlsson 1980) and isoprenaline (Eriksson & Carlsson 1982), both compounds known to cause decreased total concentrations of rat plasma LNAAs (Eriksson et al 1980, 1984). Since both amitriptyline and tryptophan are used as therapeutic agents in the treatment of depression, such a possible interaction might be of clinical importance.

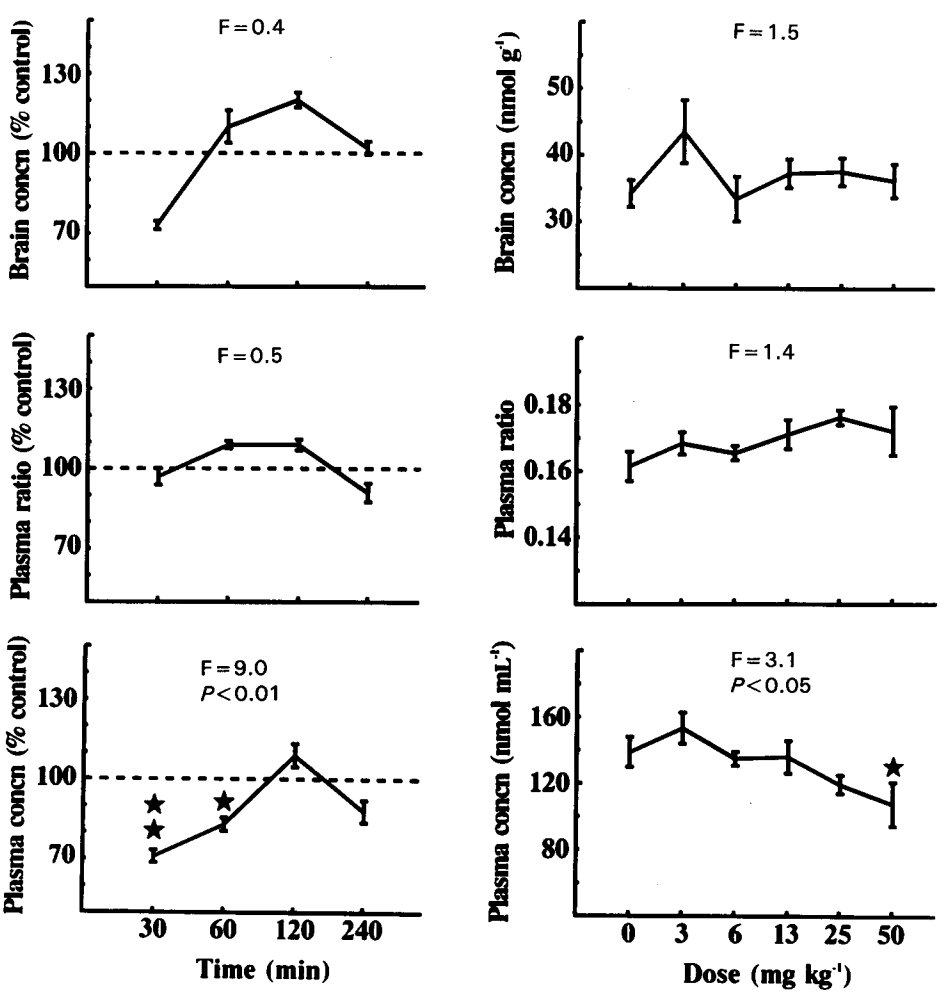

FIG. 5. Effects of amitriptyline on rat brain and plasma concentrations of isoleucine. Left panels, time course after intraperitoneal injection of 25 mg kg^{-1} amitriptyline. Right panels, effect of dose of amitriptyline at 60 min. *$P < 0.05$, **$P < 0.01$, compared with controls.

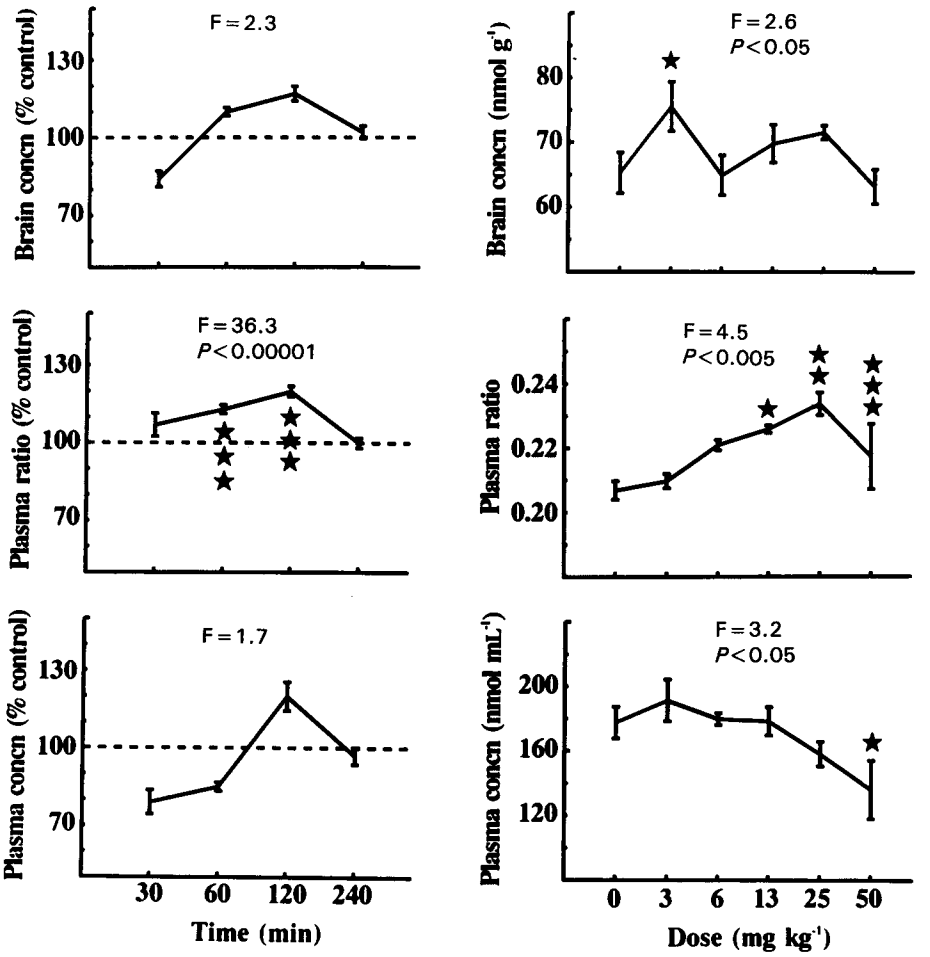

FIG. 6. Effects of amitriptyline on rat brain and plasma concentrations of leucine. Left panels, time course after intraperitoneal injection of 25 mg kg^{-1} amitriptyline. Right panels, effect of dose of amitriptyline at 60 min. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$ compared with controls.

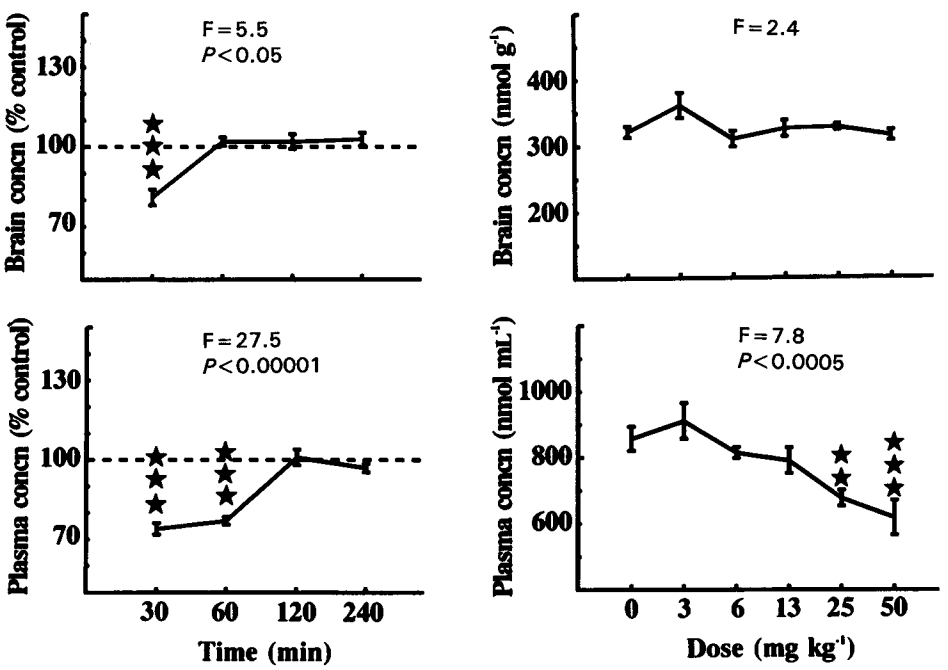

FIG. 7. Effects of amitriptyline on rat brain and plasma total concentrations of large neutral amino acids. Left panels, time course after intraperitoneal injection of 25 mg kg^{-1} amitriptyline. Right panels, effect of dose of amitriptyline at 60 min. **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$ compared with controls.

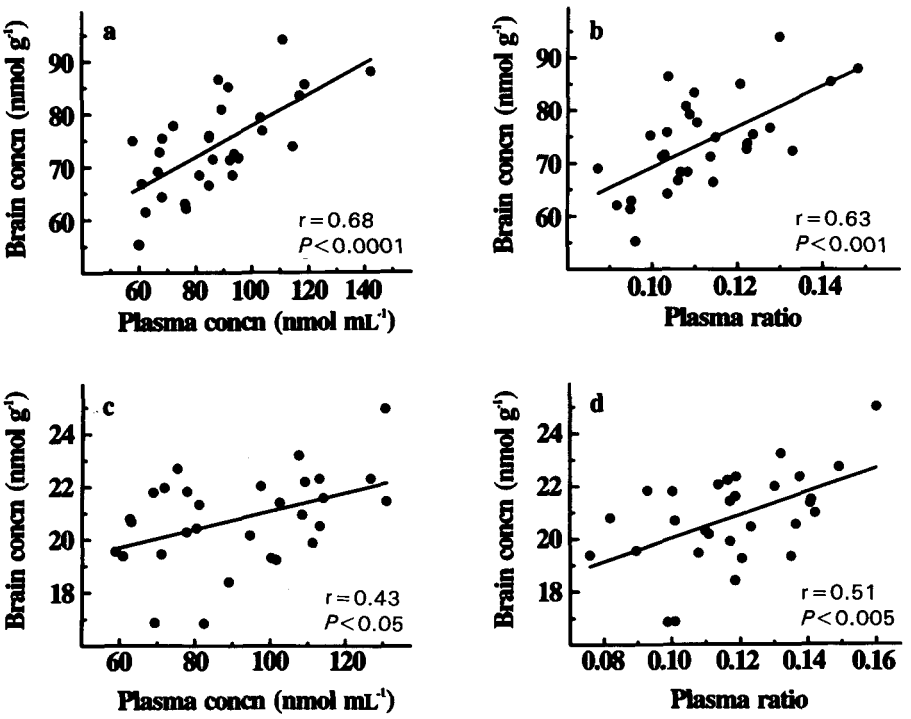

FIG. 8. Correlations between the concentrations of amino acids in plasma and brain, after treatment with amitriptyline. Upper panels, tyrosine. Lower panels, tryptophan.

Acknowledgements

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