Psychopharmacology (1990) 102:553–556

Psychopharmacology

Self-inhibiting action of nortriptyline's anti-immobility effect at high plasma and brain levels in mice

Irismar Reis de Oliveira[1,4], Bertrand Diquet[2], Véronique Van der Meersch[2], Roland Dardennes[1], Jean Gonidec[3], and Pedro Antônio do Prado-Lima[1,5]

[1] Clinique des Maladies Mentales et de l'Encéphale, 100 Rue de la Santé, F–75014 Paris, France
[2] Laboratoire de Pharmacocinétique, Groupe Hospitalier Pitié-Salpêtrière, 47 Boulevard de l'Hôpital, F–75013 Paris, France
[3] Laboratoires Delagrange, 1 Rue Pierre Brossolette, F–91380 Chilly-Mazarin, France
[4] Departamento de Farmacologia e Fisiologia, ICS, Universidade Federal da Bahia, Vale do Canela, 40140, Salvador, Bahia, Brasil
[5] Departamento de Farmacologia, Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, Rua Sarmento Leite, 245, Porto Alegre, RS, Brasil

Received January 18, 1990 / Final version May 29, 1990

Abstract. Animals were treated acutely with 0, 2.5, 5, 10, 20 and 40 mg/kg nortriptyline (NT) 30 min before the tail suspension test (TST). They were sacrificed after test for evaluation of plasma and brain levels of NT. The anti-immobility effect increased with increasing doses and concentrations of the drug, reaching statistical significance ($P < 0.01$, Dunnett test) at a dose of 20 mg/kg, 865 ng/ml in plasma and 11 µg/g in brain tissue. The anti-immobility effect was, however, blocked with the highest, non-toxic, concentrations. Results seem to indicate a biphasic curvilinear relationship between plasma and brain levels of NT and behaviour in mice.

Key words: Nortriptyline – Plasma levels – Brain levels – Tail suspension test – Mice

Since the classical pioneer studies of Åsberg et al. (1971) first found evidence of a biphasic curvilinear relationship between plasma levels of nortriptyline (NT) and clinical outcome, many other studies have been conducted with this tricyclic antidepressant (TCA) in man (De Oliveira et al. 1989c). Among existing nortriptyline studies that we could find ($n = 21$), half suggested an inhibitory effect of higher plasma levels of NT on its therapeutic effect.

Imipramine (IMI) has also been suggested to present a lower concentration limit, below which effects may be absent or poor. Although an upper limit for therapeutic effect was not found, it is usually arbitrarily set in the order of 250 ng/ml imipramine + desipramine to avoid potential toxicity (Task Force on the Use of Laboratory Tests in Psychiatry 1985).

In animal studies, the relationship between brain concentrations of the drug and behaviour has been studied by Poncelet et al. (1986) and by Mancinelli et al. (1987) using the swimming test (see Porsolt et al. 1978), and also by de Oliveira et al. (1989a, b) using the tail suspension test (see Stéru et al. 1985).

The studies by Poncelet et al. (1986) and by Mancinelli et al. (1987) involved desipramine (DMI), which in human studies gives results that are even more controversial than with NT and IMI. They did not show any clear evidence of a relationship between brain concentrations of DMI and behavioural effect.

In our study (De Oliveira et al. 1989a, b), higher levels of IMI and its demethylated metabolite DMI in brain and in blood did not impair the anti-immobility effect in mice, as demonstrated by the TST. These findings suggested a linear or sigmoid relationship between antidepressant concentrations and biological response, which is in agreement with most of the results found in human subjects treated with IMI.

The purpose of the present report was to investigate if higher brain and plasma concentrations of NT inhibited their own pharmacological effect, as has been suggested in depressed patients. Furthermore, we know of no other studies employing NT and this animal model of depression in relation to drug concentrations.

Materials and methods

Animals. Naive male Swiss mice weighing 28–30 g were used. They were housed 10–12 per cage, at room temperature 21 ± 1° C. Food and water were provided ad libitum. Animals were kept in an artificial 12-h light-dark cycle (6 a.m.: lights on, 6 p.m.: lights off).

Offprint requests to: I. Reis de Oliveira, Departamento de Farmacologia e Fisiologia, ICS, Universidade Federal da Bahia, Vale do Canela, 40140, Salvador, Bahia, Brasil

Table 1. Nortriptyline concentrations and duration of immobility at different dose regimens. Differences from controls were assessed statisticaly using the Dunnett test (two-tailed). Values represent means and SEM

Dose (mg/kg)	*n*	Brain levels (μg/g)	Plasma levels (ng/ml)	Duration of immobility	
				Seconds	*P*
0	22	–	–	74.14 (10.60)	–
2.5	10	1.14 (0.11)	135.70 (14.23)	64.40 (18.44)	ns
5	10	2.21 (0.11)	316.30 (21.14)	36.80 (11.22)	ns
10	10	5.40 (0.72)	475.10 (28.72)	38.70 (10.80)	ns
20	10	11.39 (1.24)	864.50 (69.73)	23.90 (7.04)	<0.01
40	10	32.04 (2.20)	1635.90 (116.65)	68.90 (12.18)	ns

ns = non-significant

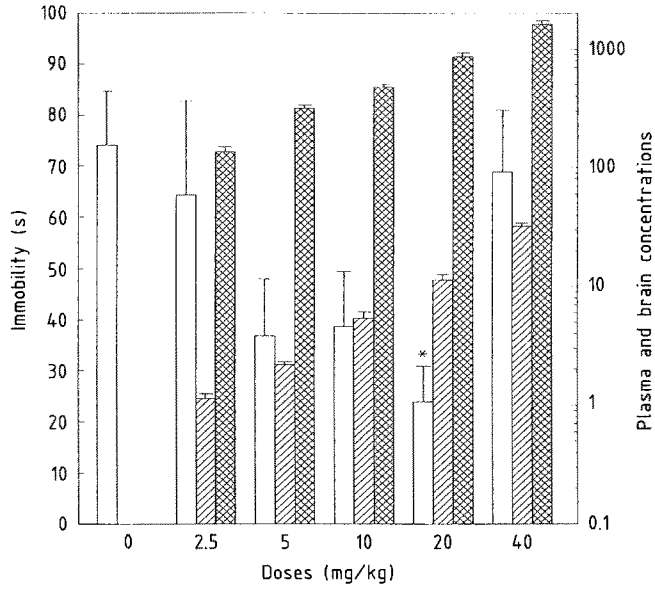

Fig. 1. Effect of different doses and concentrations of nortriptyline hydrochloride on the time of immobility in the 6-min tail suspension test. Means and SEM are given. * $P<0.01$ (Dunnett test). □ TST; ▨ brain (μg/g); ▩ plasma (ng/ml)

Drug treatment. Seventy-two animals were randomly divided into five separate groups receiving nortriptyline hydrochloride ($n=10$) and one control group receiving vehicle ($n=22$). Injections were given intraperitoneally, under blind conditions, at the doses shown in Table 1.

Testing procedure. Animals were subjected to the tail suspension test (TST), in which a mouse is suspended by the tail from a lever (Stéru et al. 1985). Meanwhile, the movements of the animal were recorded. Duration of immobility was measured 30 min after drug administration. The total duration of the test was 6 min, in which periods of agitation and immobility were observed. Measurements were taken under blind conditions. TST was performed on a Beckman polygraph connected to Narco GP A–10 integrators. Experiments were carried out between 1 and 6 p.m.

Brain and blood drug level assays. The animals were sacrificed by decapitation 1 min after TST. Blood was collected in dry heparinized tubes and centrifuged to obtain plasma. Brains were immediately removed. Plasma and brains were stored frozen at $-20°$ C until assayed. NT concentration was assessed by high performance liquid chromatography (HPLC) according to the method of Diquet et al. (1983, modified). Plasma and brain tissue from the control group were used for calibration curve preparation. Concentrations of NT were expressed in terms of ng/ml plasma and μg/g brain tissue. All samples were measured by laboratory personnel blind to protocol.

Statistics. Comparisons of the mean duration of immobility (in seconds) between different treatment groups and controls were performed by using Dunnett test (Dunnett 1964). Correlations between brain and plasma concentration of NT were performed.

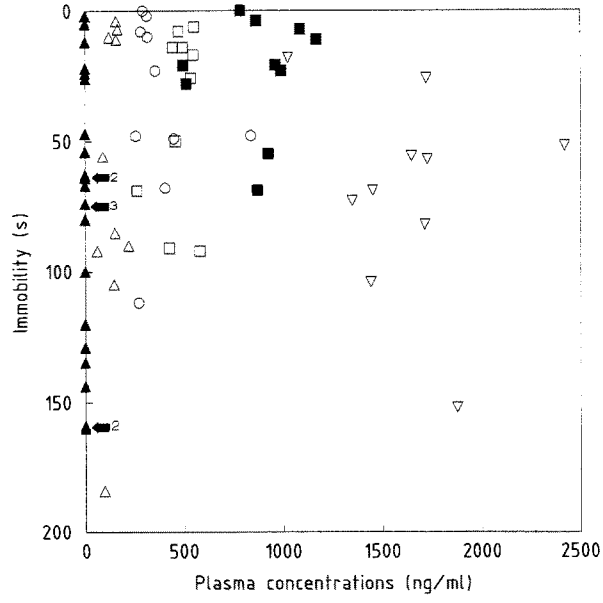

Fig. 2. Relationship of plasma concentrations of nortriptyline hydrochloride and immobility at different doses in the 6-min tail suspension test. Numbers beside arrows indicate animals presenting the same duration of immobility. ▲ = 0; △ = 2.5; ○ = 5; □ = 10; ■ = 20; ▽ = 40 mg/kg

Results

Table 1 and Figs. 1–3 show that increasing doses and concentrations of NT reduced the duration of immobility in mice, but statistical significance was reached only in the 20 mg/kg, group ($P<0{,}01$). Interestingly, a dose of 40 mg/kg produced plasma and brain concentrations that brought immobility to the level of the control group, suggesting a biphasic dose and/or concentration-effect relationship.

We found a positive significant linear correlation between plasma and brain levels of NT ($r=0.95$; $df=48$; $P<0.001$), which can be seen in Fig. 1. Controls were not included in these computations.

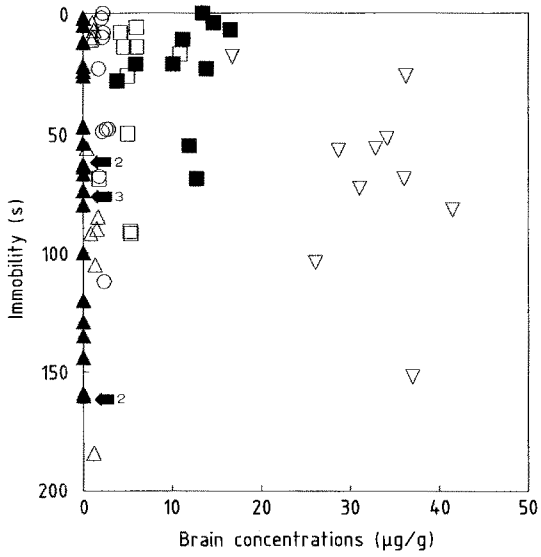

Fig. 3. Relationship of brain concentrations of nortriptyline hydrochloride and immobility at different doses in the 6-min tail suspension test. Numbers beside arrows indicate animals presenting the same duration of immobility. ▲=0; △=2.5; ○=5; □=10; ■=20; ▽=40 mg/kg

Discussion

Low TCA doses had previously been suggested to potentiate peripheral adrenergic effects in animal experiments, but effects tended to disappear with higher doses (Haefely et al. 1964). It was hypothesized that a phenothiazine-like blockade of the monoaminergic neurons would be added to the blockade of monoamine reuptake thought to be related to the antidepressant action (Åsberg et al. 1971). Subsequent clinical investigations involving NT showed that the antidepressant effect decreased at higher plasma levels (Åsberg et al. 1971; Kragh-Sørensen et al. 1976).

The mechanism by which this blockade occurs needs further elucidation. Hall and Ogren (1981) suggest that the direct activity on several receptors could be part of the mechanism by which the antidepressant drugs produce adaptative changes in various transmitter systems. In their experiments NT was the most potent antidepressant drug on [^{3}H]5–HT binding, followed by mianserin and amitriptyline. NT also showed some affinity for alpha-1 and H–1 receptors. It is possible that the effects of these receptors, in increasing the concentrations of NT, might be responsible for the results observed in our experiments.

There has been some recent evidence that hydroxy-metabolites might be involved in this self-inhibiting effect of higher concentrations of NT. In a recent publication, Young et al. (1988) were unable to demonstrate any relationship between plasma levels of NT and clinical outcome in elderly patients but showed that patients presenting higher plasma levels of E–10–OH-nortriptyline did not respond or responded poorly to treatment. They proposed that 1) an inhibitory effect of high plasma E–10–OH–NT on efficacy might be related to its lower pharmacologic potency compared to NT; 2) a competitive mechanism involving E–10–OH–NT might contribute to the curvilinear relationship between plasma NT alone and efficacy at a single stable dose in younger patients; 3) the influence of high plasma E–10–OH–NT might further depend on the concentration of NT.

Our study was designed to investigate the existence of inhibition with higher concentrations of NT upon its own initial effect, as it is the TCA for which a biphasic curvilinear relationship has been most frequently shown (De Oliveira et al. 1989c). Unfortunately, we were not able to assess OH-metabolites in our experiments. Furthermore, they have been suggested to be pharmacologically active (Potter et al. 1979; Nelson et al. 1988). Our experiments clearly demonstrate that increasing doses and concentrations of NT reduce the duration of immobility in mice, except for the highest but non-toxic concentrations (1630 ng/ml plasma or 32 µg/g brain tissue) in which the anti-immobility effect was blocked.

In contrast to humans and rats – species considered to predominantly demethylate TCAs – hydroxylation seems to be the main TCA metabolic pathway in mice (Herrmann 1970). This animal model might therefore be considered for research into the hypothesis of OH-metabolite blockade of antidepressant effect.

Acknowledgements. We thank Dr. Denis Besançon and Miss Josette Margarit, from Delagrange Laboratories, who kindly provided laboratory and animals for experiments. Thanks are also due to Dr. Miriam Chapman for correction of the original English manuscript. Without Professor Bertrand Samuel-Lajeunesse's help, this work would have been performed with much more difficulty.

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