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European Journal of Pharmacology 382 (1999) 143–150

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Inhibition of nitric oxide synthase reduces ultrasonic vocalizations of rat pups

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Received 29 April 1999; received in revised form 9 August 1999; accepted 13 August 1999

Abstract

The present study investigated the effects of drugs acting on the brain nitric oxide pathway on ultrasonic vocalizations, body temperature and locomotion in 7–8-day-old rat pups. Both a selective neuronal nitric oxide synthase (NOS) inhibitor (7-nitroindazole) and a non-selective NOS inhibitor (nitro-L-arginine-methyl ester, L-NAME) decreased the number of ultrasonic vocalizations in a dose-dependent manner. The non-selective NOS inhibitor, L-NAME, suppressed not only ultrasonic vocalizations but also locomotion. The inactive isomer of the NOS inhibitor, nitro-D-arginine-methyl ester (D-NAME), and the biological precursor of nitric oxide, L-arginine, had no effect on ultrasonic vocalizations or locomotion. These data indicate that drugs suppressing nitric oxide synthesis produced an anxiolytic effect in rat pups. However, only the selective NOS inhibitor, 7-nitroindazole, was 'anxioselective', i.e., reduced ultrasonic vocalizations without causing sedation. Increased synthesis of nitric oxide in the brain had no apparent behavioral effect in this model.

Keywords: Nitric oxide (NO); Anxiety; Ultrasonic vocalization; Locomotion; (Rat pup)

1. Introduction

Nitric oxide is a biological messenger in the central nervous system which is synthesized from the amino acid L-arginine by the enzyme nitric oxide synthase (NOS) (Garthwaite, 1991, 1995; Moncada and Higgs, 1993). It is believed that nitric oxide is synthesized in response to the activation of NMDA receptors (Garthwaite, 1991; Zhang and Snyder, 1995). Nitric oxide is involved in a wide range of physiological and behavioral functions in the brain and has also been implicated in a number of pathological neural conditions. For example, nitric oxide has been reported to play a role in neuronal development (Garthwaite, 1991), thermoregulation (Simon, 1998; Steiner et al., 1998), nociception (Crosby et al., 1995; Rice, 1995), learning and memory (Iga et al., 1993; Kato and Zorumski, 1993; Meyer et al., 1998a,b), fear, anxiety and defensive behavior (Faria et al., 1997; Volke et al., 1997), seizures (Buisson et al., 1993a; Dzoljic et al., 1997), and neurotoxicity (Buisson et al., 1993b; Verrecchia et al., 1994; Ali and Itzhak, 1998).

Several findings indicate that nitric oxide plays an important role in anxiety-related behaviors. First, high concentrations of neuronal NOS can be found in brain regions involved in the modulation of anxiety and defensive behavior, including the amygdala, hypothalamus, periaqueductal grey and pedunculopontine tegmental nucleus (Vincent and Kimura, 1992). Second, exposure to stressful stimuli induces the activation of nitric oxide-producing neurons in those brain regions (Krukoff and Khalili, 1997). Third, the blockade of nitric oxide synthesis results in decreased anxiety-like behavior in several animal models of anxiety. For example, administration of NOS inhibitors results in increased open-arm exploration in the elevated plus maze (Guimaraes et al., 1991, 1994; Volke et al., 1995, 1997; De Oliveira et al., 1997), increased number of light box entries and light box exploration in the light/dark transition box (Volke et al., 1997), increased social investigation in the social interaction test (Volke et al., 1997), and decreased number of ultrasonic calls in the rat pup ultrasonic vocalization test (Campbell et al., 1999).

L-arginine is the biological precursor of nitric oxide (Garthwaite, 1991). Although systemic administration of L-arginine increases nitric oxide levels in the cerebellum, as measured ex vivo, 60 min after drug administration (Salter et al., 1996), the in vivo effects of L-arginine on

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00595-6

anxiety-related behaviors remain unknown. L-arginine antagonizes the anxiolytic effects of NOS inhibitors (Guimaraes et al., 1994; De Oliveira et al., 1997) but neither systemic nor i.c.v. administration of L-arginine produces behavioral changes indicative of increased anxiety in the elevated plus maze (Faria et al., 1997; Volke et al., 1997) or in the rat pup ultrasonic vocalization test (Campbell et al., 1999).

Infant mammals, when isolated from their mother and littermates, emit ultrasonic vocalizations and measuring these vocalizations has been used as a model of anxiety (Gardner, 1985; Winslow and Insel, 1991). The rodent pup ultrasonic vocalization model is sensitive to both the anxiolytic and anxiogenic effects of drugs acting via central benzodiazepine receptors (Gardner, 1985; Nastiti et al., 1991). This model is also sensitive to the effects of drugs acting at the NMDA receptors (Winslow et al., 1990), cannabinoid receptors (McGregor et al., 1996), opioid receptors (Barr et al., 1994), dopamine receptors (Dastur et al., 1999), and serotonergic receptors (Winslow and Insel, 1990; Klint and Andersson, 1994).

Although inhibition of nitric oxide synthesis has been shown to reduce anxiety in animal models using adult rats, little data are available on the effects of nitric oxide modulation on behavioral measures of anxiety in infant rodents. The only study that utilized the rat pup ultrasonic vocalization model of anxiety examined effects of only the non-selective NOS inhibitor (L-NAME) and one dose of the biological precursor, L-arginine (Campbell et al., 1999). The aim of the present study, therefore, was to study the

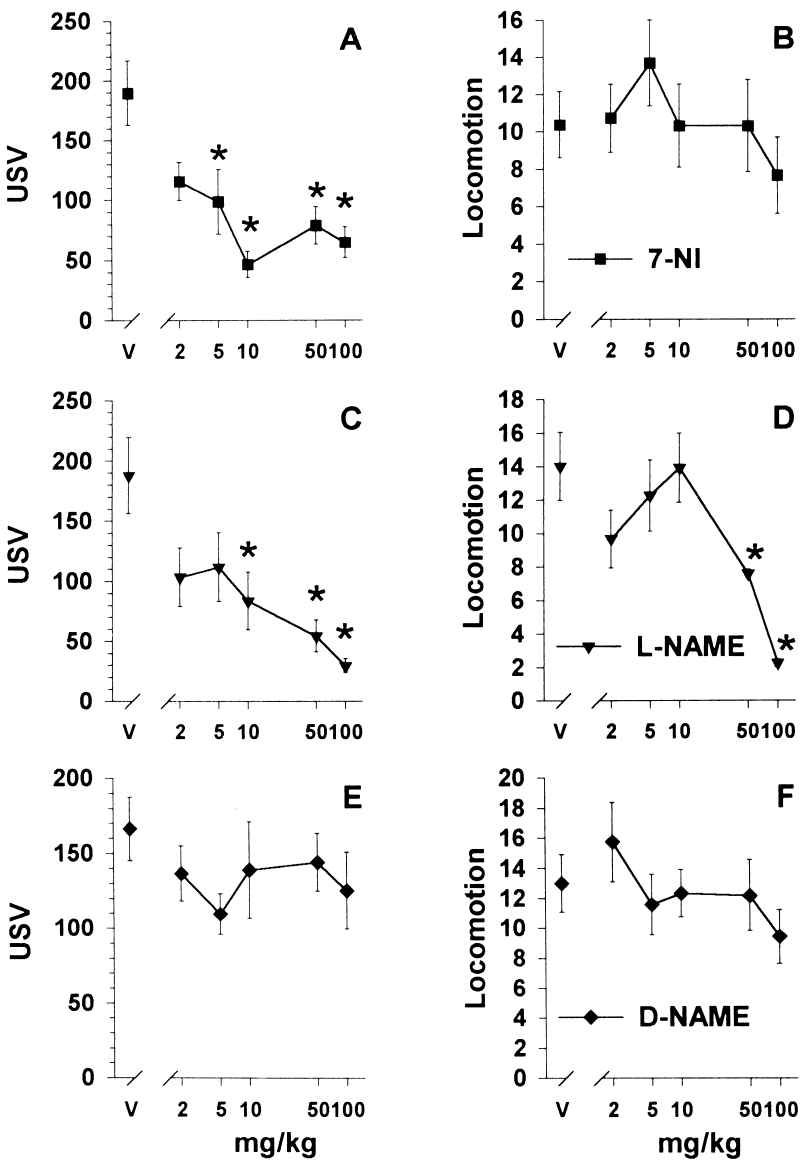

Fig. 1. Effects of NOS inhibitors on ultrasonic vocalizations (USV) and line crossing (Locomotion) in 7–8 day old rat pups: the selective neuronal NOS inhibitor, 7-nitroindazole (A, B), the non-selective NOS inhibitor, L-NAME (C, D), and the inactive isomer of neuronal NOS inhibitors, D-NAME (E, F). The figures show mean number ± S.E.M. $^{*}P < 0.05$ compared to vehicle (V) group.

dose–response effects of the nitric oxide precursor (L-arginine) and a number of NOS inhibitors on the ultrasonic vocalizations, locomotion and body temperature in rat pups. We wanted to confirm that changes in neuronal NOS activity found in vitro in both adult (Salter et al., 1996) and infant rodents (Prickaerts et al., 1998) would result in behavioral changes indicative of decreased or increased anxiety. Thus, the following drugs were administered at a wide range of doses: the biological precursor of nitric oxide, L-arginine; the selective neuronal NOS inhibitor, 7-nitroindazole; the non-selective NOS inhibitors, nitro-L-arginine-methyl ester (L-NAME); and the inactive isomer of the NOS inhibitor, nitro-D-arginine-methyl ester (D-NAME). Our hypothesis was that the reduced nitric oxide synthesis caused by NOS inhibitors would reduce isolation-induced ultrasonic vocalizations while the increased nitric oxide synthesis produced by L-arginine would increase ultrasonic vocalizations in this model.

2. Method

2.1. Subjects

The offspring of mated pairs of Long–Evans hooded rats originally purchased from Charles River, Canada (St. Constant, Quebec) and bred in the Psychology Department, Dalhousie University were used as subjects. The adults were housed in standard Plexiglas cages ($23 \times 45 \times 25$ cm) in a vivarium with room temperature of 22 ± 1°C. They were kept on a reversed 12:12 h light–dark cycle with lights on from 2100 to 0900 h. Pairs of rats were mated for 10 days and then the male was removed. Purina rat chow and water were available ad libitum.

Following birth, each litter was housed with its mother in a Plexiglas cage with wood shavings for bedding and shredded paper for nesting material. Bedding material was not changed from the time of birth until a day after the testing day. Litters were culled to 10 pups on day 4 (parturition as day 0) and litters containing fewer than 10 pups received additional pups from bigger litters on day 4. All pups were tested once when 7–8 days of age. The average weight ($\pm$standard deviation), when tested, was 15.43 ± 2.64 g.

2.2. Apparatus

Testing for ultrasonic vocalizations and locomotor activity took place in a Plexiglas chamber ($26 \times 15 \times 12$ cm) with the floor divided into eight rectangles (6×7.5 cm). Ultrasonic vocalizations were recorded via an ultrasonic microphone connected to a SM2 bat detector (Ultrasound Advice, UK). The broadband output of the bat detector was fed into a custom-built four-channel digitizer based on that designed by Harrison and Holman (1978). This digitizer contained four variable band pass filters that were set to 28, 36, 44 and 52 kHz. When input was detected at one of these frequencies, the digitizer produced a pulse for the duration of the signal. The output of the digitizer was connected via a terminal panel and interface card (Strawberry Tree, Sunnyvale, CA, USA) to a Macintosh 2cx computer on which a custom program written using the Strawberry Tree Workbench Mac software (McGregor, 1996) recorded the occurrence of each ultrasonic vocalization on a minute-by-minute basis.

2.3. Drugs

7-Nitroindazole (Sigma) was suspended in a solution of dimethyl sulfoxide (DMSO), propylene glycol and distilled water (1:3:6). Nitro-L-arginine-methyl ester (L-NAME, Sigma), nitro-D-arginine-methyl ester (D-NAME, Sigma) and L-arginine (Sigma) were dissolved in saline. All drugs were injected subcutaneously in a volume of 2 ml/kg. 7-nitroindazole, L-NAME and D-NAME were administered 30 min prior to testing. L-arginine was administered either 30 min or 60 min prior to testing. Control groups were treated with the vehicle for the particular drugs.

2.4. Procedure

On the experimental day, pups were transported together with their mother in their home cages to the experimental room and left undisturbed for at least 15 min. Each pup then had its basal temperature (pre-treatment temperature) measured by placing a thermocouple, connected to a Physitemp Model B Thermometer, under the left armpit. Pups were then weighed, marked with a nontoxic perma-

Table 1
The effects of NOS inhibitors, 7-nitroindazole and L-NAME, and the inactive isomer, D-NAME, on body temperature. Data are presented as mean $\pm$ S.E.M.

Drug	Dose (mg/kg)	Pre-treatment temperature	Post-treatment temperature
(A) 7-nitroindazole	0	33.9 ± 0.5	33.4 ± 0.5
	2	33.0 ± 0.6	33.3 ± 0.6
	5	33.7 ± 0.6	33.6 ± 0.5
	10	33.2 ± 0.5	33.2 ± 0.7
	50	33.8 ± 0.5	34.1 ± 0.5
	100	34.1 ± 0.4	33.9 ± 0.4
(B) L-NAME	0	34.8 ± 0.2	34.3 ± 0.2
	2	34.1 ± 0.3	34.2 ± 0.2
	5	34.7 ± 0.2	34.1 ± 0.3[a]
	10	34.5 ± 0.3	34.3 ± 0.2
	50	34.5 ± 0.3	33.9 ± 0.3
	100	34.4 ± 0.3	33.8 ± 0.2
(C) D-NAME	0	35.1 ± 0.1	35.2 ± 0.2
	2	34.9 ± 0.2	35.1 ± 0.1
	5	35.0 ± 0.3	35.0 ± 0.2
	10	35.2 ± 0.1	34.8 ± 0.1[b]
	50	35.3 ± 0.1	35.0 ± 0.1[b]
	100	34.9 ± 0.2	34.8 ± 0.2

[a] < 0.05.
[b] < 0.01 compared to pre-treatment measure (paired t-test).

nent marker and individually placed into the testing chamber for a 1-min ultrasound screening. Pups that did not emit ultrasounds within 60 s were excluded from the experiment. Most of pups emitted ultrasounds and were assigned to a treatment based on a split-litter design (one to two or two to three pups per litter for each of the treatment groups depending on the total number of groups) and immediately injected either with the vehicle or one of the test drug doses. After injections, pups were returned to their home cage nests with their mother and littermates for 30 min (7-nitroindazole, L-NAME, D-NAME, L-arginine) or 60 min (L-arginine).

From the 371 pups screened for ultrasonic vocalization, 312 emitted ultrasounds during the 1-min screening period and were allocated to 26 experimental groups based on a split-litter design, with 11–13 pups per group. Since different vehicles were used to dissolve tested drugs, there was a vehicle treated group for each drug tested, i.e., five vehicle treated groups.

After the appropriate post-injection interval had elapsed, pups were removed from the nest and their temperature was measured a second time (post-treatment temperature). Pups were then individually placed in the center of the test chamber for 3 min. The number of ultrasonic vocalizations emitted was recorded automatically and the number of lines crossed (with two paws) was measured by an observer sitting quietly in the testing room.

2.5. Data analysis

Data on ultrasonic vocalizations and locomotion that showed homogeneity of variance were analyzed using a one-way analysis of variance (StatView). Non-homogenous data were transformed using square root transformation before analysis. Data that did not meet the criteria for parametric statistics after transformation were analyzed using a non-parametric Kruskall–Wallis test. Multiple comparisons were carried out using parametric or non-parametric versions of Dunn's test (Glantz, 1992). Changes in body temperature within each experimental group were analyzed using a paired *t*-test or non-parametric Wilcoxon test.

3. Results

3.1. Baseline responding

The mean body temperature before drug administration was 34.82°C (range of groups: 33.88–35.34°C) and after

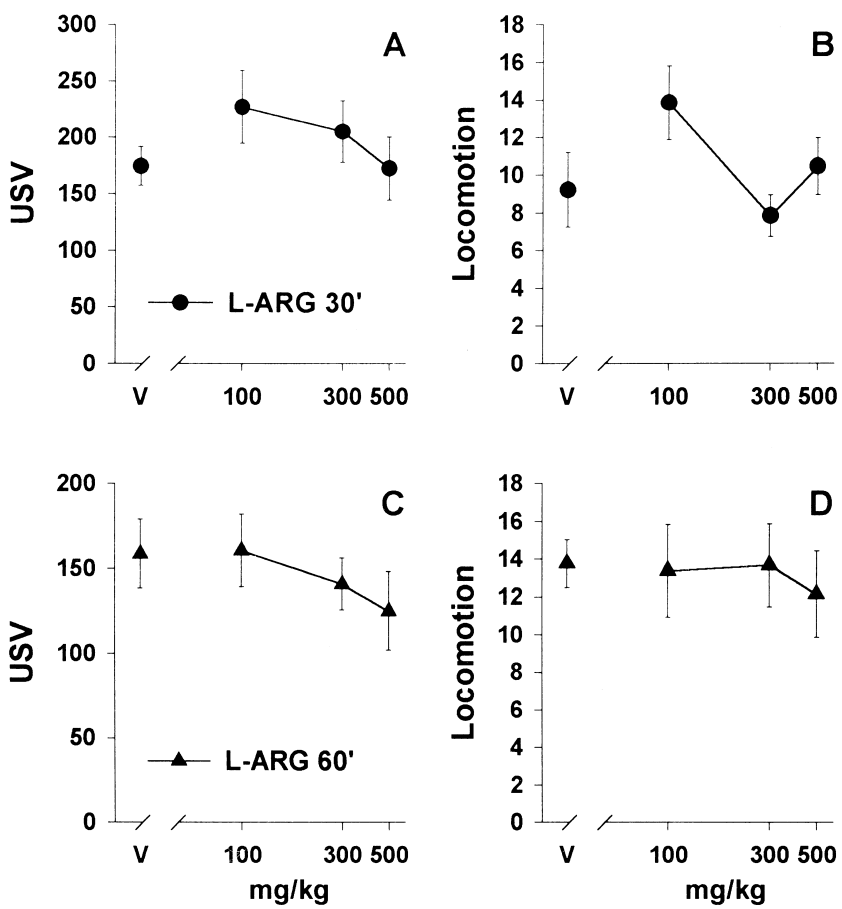

Fig. 2. Effects of the biological precursor of nitric oxide, L-arginine, on the number of ultrasonic vocalizations (USV) and the number of line crossing (Locomotion) by rat pups given the vehicle (V) or 100, 300 or 500 mg/kg SC of L-arginine administered 30 min (A, B) or 60 min prior to testing (C, D). The figures show mean number ± S.E.M.

Table 2
The effect of the biological precursor of nitric oxide, L-arginine, on body temperature. L-arginine was administered 30 min (left column) or 60 min (right column) prior to testing. Data are presented as mean ± S.E.M.

Dose (mg/kg)	Temperature (30 min after administration)		Temperature (60 min after administration)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
0	35.0 ± 0.2	35.1 ± 0.2	35.3 ± 0.2	35.5 ± 0.2
100	35.2 ± 0.2	35.0 ± 0.1	35.6 ± 0.2	35.7 ± 0.2
300	35.0 ± 0.2	35.0 ± 0.1	35.0 ± 0.2	35.3 ± 0.2[a]
500	35.1 ± 0.2	34.9 ± 0.2	35.4 ± 0.2	35.1 ± 0.2

[a] < 0.05 compared to pre-treatment measure (paired *t*-test).

drug administration, mean body temperature was 34.67°C (range of groups: 33.39–35.51°C). There was a difference among vehicle treated groups in pre-treatment body temperature ($H(4) = 10.6$, $P < 0.05$). Post-hoc multiple comparison (Dunn's) revealed a difference in basal body temperature (pre-treatment) between the vehicle treated group for 7-nitroindazole (mean ± S.E.M.: 33.88 ± 0.50°C) and vehicle treated group for L-arginine (60 min) (mean ± S.E.M.: 35.34 ± 0.17°C; $P < 0.05$).

During the 3-min test period, vehicle treated rat pups emitted a mean of 165.02 ultrasounds (range of groups: 158.85–189.77) and crossed a mean of 12.02 grids (range of groups: 9.23–14.0). There was no significant difference among vehicle treated groups in the number of ultrasonic vocalizations ($F(4, 57) = 0.451$, $P = 0.771$) or lines crossed ($F(4, 57) = 1.392$, $P = 0.248$).

3.2. NOS inhibitors

The selective neuronal NOS inhibitor, 7-nitroindazole, significantly suppressed ultrasonic vocalizations in rat pups ($H(5) = 22.3$, $P < 0.001$). Post-hoc multiple analyses (non-parametric Dunn's test) revealed significant differences between the vehicle treated controls and experimental groups starting at 5 mg/kg of 7-nitroindazole (Fig. 1A). The number of grids crossed (locomotion) was not significantly decreased at any dose of 7-nitroindazole used ($F(5, 67) = 0.836$, NS; Fig. 1B), although there was a trend toward decreased locomotion at the highest dose (100 mg/kg). There was no effect of 7-nitroindazole on post-treatment body temperature (Table 1).

The non-selective NOS inhibitor, nitro-L-arginine-methyl ester (L-NAME), at doses of 10, 50 and 100 mg/kg SC also decreased the number of ultrasonic vocalization in rat pups separated from their mother and littermates ($H(5) = 20.8$, $P < 0.001$; Fig. 1C). Low doses of L-NAME (2 and 5 mg/kg SC) had no significant effect on ultrasonic vocalizations. L-NAME at the highest doses (50 and 100 mg/kg) also suppressed locomotion ($F(5, 66) = 7.726$, $P < 0.0001$; Fig. 1D). The 5 mg/kg of L-NAME decreased the body temperature of pups ($t(11) = 2.47$, $P < 0.05$) while other doses and the vehicle (saline) had no effect (Table 1).

The inactive isomer of the NOS inhibitor, nitro-D-arginine-methyl ester (D-NAME), had no effect at any dose on the number of ultrasonic vocalizations ($F(5, 66) = 0.712$, NS, Fig. 1E) or on locomotion ($F(5, 66) = 0.973$, NS; Fig. 1F). D-NAME, however, reduced body temperature at doses of 10 mg/kg ($t(11) = 3.47$, $P < 0.01$) and 50 mg/kg ($t(11) = 3.40$, $P < 0.01$; Table 1).

3.3. L-Arginine, a biological precursor of nitric oxide

L-Arginine had no effect on the number of ultrasonic vocalizations either 30 min ($F(3, 48) = 1.469$, NS; Fig. 2A) or 60 min after drug administration ($F(3, 47) = 0.794$, NS; Fig. 2C). Locomotion was not significantly changed by any dose of L-arginine administered 30 min ($F(3, 48) = 2.326$, NS; Fig. 2B) or 60 min prior to testing ($F(3, 47) = 0.128$, NS; Fig. 2D). L-arginine administered 30 min prior to testing had no effect on body temperature (Table 2). However, 300 mg/kg of L-arginine administered 60 min prior to testing increased body temperature in pups ($t(11) = 2.38$, $P < 0.05$; Table 2).

4. Discussion

In the rat pup ultrasonic vocalization model of anxiety, a decreased number of ultrasonic vocalizations emitted by the pup indicates a decrease in anxiety while an increased number of calls indicates increased anxiety (Gardner, 1985; Winslow and Insel, 1991). Our results show that NOS inhibitors reduced ultrasonic vocalizations, producing an anxiolytic effect in rat pups. The selective neuronal NOS inhibitor, 7-nitroindazole, appeared to be 'anxioselective' in that it had an anxiolytic effect without reducing locomotor activity. The non-selective NOS inhibitor, nitro-L-arginine-methyl ester (L-NAME), not only decreased ultrasonic vocalizations but also, at higher doses, decreased locomotion. The inactive isomer, nitro-D-arginine-methyl ester (D-NAME), had no effect on ultrasonic vocalizations or locomotion and thus, confirmed that the anxiolytic effect of NOS inhibitors was due to the inhibition of nitric oxide synthesis. The biological precursor of nitric oxide, L-arginine, failed to produce any behavioral effects in this model.

Both NOS inhibitors tested in our study decreased the number of ultrasonic vocalizations in rat pups which indicates that they possess anxiolytic effects. Our results are consistent with those of Campbell et al. (1999) that found decreased ultrasonic vocalizations in 10–11-day-old pups after administration of the non-selective NOS inhibitor, L-NAME. Our data are also in agreement with previous studies that found anxiolytic effects of 7-nitroindazole and L-NAME in the elevated plus maze, the open field test, and the social interaction test, using adult rats (Guimaraes et al., 1994; Volke et al., 1995, 1997; Faria et al., 1997). However, the ultrasonic vocalization model appeared to be more sensitive in detecting the anxiolytic properties of NOS inhibitors, particularly that of 7-nitraindazole, than models based on exploration (Lister, 1990). 7-Nitroindazole produced an anxiolytic effect at 5 mg/kg in the rat pup ultrasonic vocalization model while it was ineffective in the elevated plus maze test up to 40 mg/kg in adult rats and up to 80 mg/kg in adult mice (Volke et al., 1997). Thus, our results confirmed not only the anxiolytic properties of NOS inhibitors but also the high sensitivity of the rodent pup ultrasonic vocalization model of anxiety for drug testing.

The 'anxioselectivity' of a drug is characterized by its anxiolytic effect without producing motor impairment (Mosconi et al., 1993). Our results showed that only the selective neuronal NOS inhibitor, 7-nitroindazole, decreased ultrasonic calls without the concomitant suppression of line crossing. This is contrary to other studies that found the 7-nitroindazole to be sedative (Starr and Starr, 1995; Volke et al., 1997). However, the results of those studies are inconsistent. In mice, 7-nitroindazole decreased open field locomotion at 25 and 50 mg/kg in one study (Starr and Starr, 1995) but not up to 80 mg/kg in another study (Volke et al., 1997), although the same species, test and drug administration interval were used. In rats, 7-nitroindazole decreased open field locomotion at 10 mg/kg if administered 40 min prior to testing but not up to 90 mg/kg if administered 60 min prior to testing (Volke et al., 1997). We did not find a significant sedative effect of 7-nitroindazole in rat pups in our model although there was a trend toward decreased locomotion at 100 mg/kg (see Fig. 1B). L-NAME suppressed both the number of ultrasonic calls and, at higher doses, of line crossing. Only the intermediate dose of L-NAME (10 mg/kg) produced an anxiolytic effect without suppressing locomotion, which is in agreement with results from other animal models of anxiety (Volke et al., 1995). Higher doses of L-NAME (50 and 100 mg/kg) suppressed both ultrasonic vocalizations and locomotion.

L-arginine is the biological precursor of nitric oxide and was reported to increase nitric oxide levels in the cerebellum ex vivo 60 min after drug administration (150 and 300 mg/kg IP) (Salter et al., 1996). In vivo, L-arginine antagonized behavioral effects of various NOS inhibitors (Quock and Nguyen, 1992; Buisson et al., 1993b; Guimaraes et al., 1994). Although there was a slight trend for increased numbers of ultrasonic vocalizations and line crossing 30 min after administration of L-arginine (100 mg/kg), it failed to produce marked effects on ultrasonic vocalizations or locomotion in our study as well as in the study of Campbell et al. (1999). In addition, L-arginine produced no anxiogenic effects in other animal models of anxiety (De Oliveira et al., 1997; Faria et al., 1997; Volke et al., 1997). This suggests that the effects of nitric oxide on anxiety and defensive behavior may be due to interactions with other neurochemicals. In this regard, it is interesting that nitric oxide interacts with, modulates or is co-localized with a number of neurotransmitters that are known to play a role in anxiety and defensive behavior including glutamate (Garthwaite, 1991; Zhang and Snyder, 1995), noradrenaline (Kandasamy, 1994), serotonin (Pögün and Kuhar, 1994; Cespuglio et al., 1998) and γ-aminobutyric acid (Cespuglio et al., 1998).

Although the nitric oxide pathway may play a role in thermoregulation (Simon, 1998), our results showed that there was no effect of the selective neuronal NOS inhibitor, 7-nitroindazole, on body temperature. The non-selective NOS inhibitor, L-NAME, and its inactive isomer, D-NAME, decreased body temperature at intermediate doses. On the other hand, a dose of 300 mg/kg of the nitric oxide precursor L-arginine increased body temperature 60 min after drug administration. It remains unclear why the non-selective NOS inhibitor, but not the selective neuronal NOS inhibitor, affected body temperature. As reviewed by Simon (1998), the exact role of NOS inhibitors and nitric oxide precursor in thermoregulation has not been determined. Still, our results are in agreement with studies on adult rodents that showed that the selective NOS inhibitor has no effect on body temperature (Demas et al., 1997; Ali and Itzhak, 1998) while the non-selective NOS inhibitor decreases it (Steiner et al., 1998; Campbell et al., 1999).

Nitric oxide synthesis in the brain occurs in response to NMDA receptor activation (Garthwaite, 1991; Zhang and Snyder, 1995). Inhibition of the catalytic enzyme (NOS) essential for nitric oxide synthesis results in decreased anxiety in rodents as does the blockade of NMDA receptors by NMDA antagonists (Bennett and Amrick, 1986; Dunn et al., 1990; Guimaraes et al., 1991; Corbett and Dunn, 1993; Fraser et al., 1996). The selective NOS inhibitor, 7-nitroindazole, appeared to be 'anxioselective', as it produced an anxiolytic effect without causing apparent motor impairment in this model. No behavioral anxiogenic effect of the biological precursor of nitric oxide, L-arginine, was observed in our study or in other studies (De Oliveira et al., 1997; Faria et al., 1997; Volke et al., 1997; Campbell et al., 1999), presumably due to the lack of retrograde effects of increased nitric oxide levels on NMDA activation. Moreover, our results showed that the model of ultrasonic vocalization in rodent pups is more sensitive in detecting anxiolytic activity than animal mod-

els of anxiety based on exploration (Lister, 1990), such as the elevated plus maze. Although the use of NOS inhibitors as potential therapies for anxiety might be limited due to their pro-aggressive (Demas et al., 1997) and learning/memory impairment properties (Meyer et al., 1998b), we conclude that drugs acting at the nitric oxide pathway might be useful in elucidating the neurobiology of anxiety.

Acknowledgements

This research was supported by an NSERC of Canada research grant to Richard E. Brown and was conducted under approval of the Dalhousie University Animal Care Committee protocol number 98-054.

References

Ali, S.F., Itzhak, Y., 1998. Effects of 7-nitroindazole, an NOS inhibitor on methamphetamine-induced dopaminergic and serotonergic neurotoxicity in mice. Ann. N.Y. Acad. Sci. 844, 122–130.

Barr, G.A., Wang, S., Carden, S., 1994. Aversive properties of the kappa opioid agonist U50,488 in the week-old rat pup. Psychopharmacology 113, 422–428.

Bennett, D.A., Amrick, C.L., 1986. 2-Amino-7-phosphonoheptanoic acid (AP7) produces discriminative stimuli and anticonflict effects similar to diazepam. Life Sci. 39, 2455–2461.

Buisson, A., Lakhmeche, N., Verrecchia, C., Plotkine, M., Boulu, R.G., 1993a. Nitric oxide: an endogenous anticonvulsant substance. NeuroReport 4, 444–446.

Buisson, A., Margaill, I., Callebert, J., Plotkine, M., Boulu, R.G., 1993b. Mechanisms involved in the neuroprotective activity of a nitric oxide synthase inhibitor during focal cerebral ischemia. J. Neurochem. 61, 690–696.

Campbell, J.O., Fogarty, J.A., Patia Spear, L., 1999. Inhibition of nitric oxide synthesis with L-NAME suppresses isolation-induced ultrasounds in rat pups. Pharmacol. Biochem. Behav. 63, 45–53.

Cespuglio, R., Burlet, S., Faradji Prevautel, H., 1998. 5-Hydroxyindoles compounds and nitric oxide voltammetric detection in the rat brain: changes occurring throughout the sleep–wake cycle. J. Neural Transm. 105, 205–215.

Corbett, R., Dunn, R.W., 1993. Effects of 5,7-dichlorokynurenic acid on conflict, social interaction and plus maze behaviors. Neuropharmacology 32, 461–466.

Crosby, G., Marota, J.J., Huang, P.L., 1995. Intact nociception-induced neuroplasticity in transgenic mice deficient in neuronal nitric oxide synthase. Neuroscience 69, 1013–1017.

Dastur, F.N., McGregor, I.S., Brown, R.E., 1999. Dopaminergic modulation of rat pup ultrasonic vocalizations. Eur. J. Pharmacol., (in press).

Demas, G.E., Eliasson, M.J., Dawson, T.M., Dawson, V.L., Kriegsfeld, L.J., Nelson, R.J., Snyder, S.H., 1997. Inhibition of neuronal nitric oxide synthase increases aggressive behavior in mice. Mol. Med. 3, 610–616.

De Oliveira, C.L., Del Bel, E.A., Guimaraes, F.S., 1997. Effects of L-NOARG on plus-maze performance in rats. Pharmacol. Biochem. Behav. 56, 55–59.

Dunn, R.W., Corbett, R., Martin, L.L., Payack, J.F., Laws-Ricker, L., Wilmot, C.A.X., Rush, D.K., Cornfeldt, M.L., Fielding, S., 1990. Preclinical anxiolytic profiles of 7189 and 8319, novel non-competitive NMDA antagonists. Prog. Clin. Biol. Res. 361, 495–512.

Dzoljic, E., De Vries, R., Dzoljic, M.R., 1997. Anticonvulsant activity of new and potent inhibitors of nitric oxide synthase. Brain Res. Bull. 43, 191–195.

Faria, M.S., Muscara, M.N., Moreno, H. Jr., Teixeira, S.A., Dias, H.B., De Oliveira, B., Graeff, F.G., De Nucci, G., 1997. Acute inhibition of nitric oxide synthesis induces anxiolysis in the plus maze test. Eur. J. Pharmacol. 323, 37–43.

Fraser, C.M., Cooke, M.J., Fisher, A., Thompson, I.D., Stone, T.W., 1996. Interactions between ifenprodil and dizocilpine on mouse behavior in models of anxiety and working memory. Eur. Neuropsychopharmacol. 6, 311–316.

Gardner, C.R., 1985. Distress vocalization in rat pups. A simple screening method for anxiolytic drugs. J. Pharmacol. Methods 14, 181–187.

Garthwaite, J., 1991. Glutamate, nitric oxide and cell–cell signalling in the nervous system. Trends Neurosci. 14, 60–67.

Garthwaite, J., 1995. Neural nitric oxide signalling. Trends Neurosci. 18, 51–52.

Glantz, S.A., 1992. Primer of Biostatistics. 3rd edn. McGraw-Hill, New York.

Guimaraes, F.S., Carobrez, A.P., De Aguiar, J.C., Graeff, F.G., 1991. Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey. Psychopharmacology 103, 91–94.

Guimaraes, F.S., De Aguiar, J.C., Del Bel, E.A., Ballejo, G., 1994. Anxiolytic effect of nitric oxide synthase inhibitors microinjected into the dorsal central grey. NeuroReport 5, 1929–1932.

Harrison, G.E., Holman, S.D., 1978. A device to enable the automatic recording of rodent ultrasonic vocalizations. Behav. Res. Meth. Instrum. 10, 689–692.

Iga, Y., Yoshioka, M., Togashi, H., Saito, H., 1993. Inhibitory action of *N*-mega-nitro-L-arginine methyl ester on in vivo long-term potentiation in the rat dentate gyrus. Eur. J. Pharmacol. 238, 395–398.

Kandasamy, S.B., 1994. Role of nitric oxide synthase, superoxide dismutase, and glutathione peroxidase in radiation-induced decrease in norepinephrine release. Ann. N.Y. Acad. Sci. 738, 163–172.

Kato, K., Zorumski, C.F., 1993. Nitric oxide inhibitors facilitate the induction of hippocampal long-term potentiation by modulating NMDA responses. J. Neurophysiol. 70, 1260–1263.

Klint, T., Andersson, G., 1994. Ultrasound vocalization is not related to corticosterone response in isolated rat pups. Pharmacol. Biochem. Behav. 47, 947–950.

Krukoff, T.L., Khalili, P., 1997. Stress-induced activation of nitric oxide-producing neurons in the rat brain. J. Comp. Neurol. 377, 509–519.

Lister, R.G., 1990. Ethologically-based animal models of anxiety disorders. Pharmacol. Ther. 46, 321–340.

McGregor, I.S., 1996. Using Strawberry Tree WorkbenchMac and Workbench PC software for data acquisition and control in the animal learning laboratory. Behav. Res. Meth. Instr. Comput. 28, 38.

McGregor, I.S., Dastur, F.N., McLellan, R.A., Brown, R.E., 1996. Cannabinoid modulation of rat pup ultrasonic vocalization. Eur. J. Pharmacol. 313, 43–49.

Meyer, R.C., Spangler, E.L., Kametani, H., Ingram, D.K., 1998a. Age-associated memory impairment. Assessing the role of nitric oxide. Ann. N.Y. Acad. Sci. 854, 307–317.

Meyer, R.C., Spangler, E.L., Patel, N., London, E.D., Ingram, D.K., 1998b. Impaired learning in rats in a 14-unit T-maze by 7-nitroindazole, a neuronal nitric oxide synthase inhibitor, is attenuated by the nitric oxide donor, molsidomine. Eur. J. Pharmacol. 341, 17–22.

Moncada, S., Higgs, A., 1993. The L-arginine–nitric oxide pathway. N. Engl. J. Med. 329, 2002–2012.

Mosconi, M., Chiamulera, C., Recchia, G., 1993. New anxiolytics in development. Int. J. Clin. Pharmacol. Res. 13, 331–344.

Nastiti, K., Benton, D., Brain, P.F., 1991. The effects of compounds acting at the benzodiazepine receptor complex on the ultrasonic calling of mouse pups. Behav. Pharmacol. 2, 121–128.

Pögün, S., Kuhar, M.J., 1994. Regulation of neurotransmitter reuptake by nitric oxide. Ann. N.Y. Acad. Sci. 738, 305–315.

Prickaerts, J., De Vente, J., Markerink Van Ittersum, M., Steinbusch, H.W., 1998. Behavioral, neurochemical and neuroanatomical effects of chronic postnatal *N*-nitro-L-arginine methyl ester treatment in neonatal and adult rats. Neuroscience 87, 181–195.

Quock, R.M., Nguyen, E., 1992. Possible involvement of nitric oxide in chlordiazepoxide-induced anxiolysis in mice. Life Sci. 51, PL255–PL260.

Rice, A.S., 1995. Topical spinal administration of a nitric oxide synthase inhibitor prevents the hyper-reflexia associated with a rat model of persistent visceral pain. Neurosci. Lett. 187, 111–114.

Salter, M., Strijbos, P.J., Neale, S., Duffy, C., Follenfant, R.L., Garthwaite, J., 1996. The nitric oxide–cyclic GMP pathway is required for nociceptive signalling at specific loci within the somatosensory pathway. Neuroscience 73, 649–655.

Simon, E., 1998. Nitric oxide as a peripheral and central mediator in temperature regulation. Amino Acids 14, 87–93.

Starr, M.S., Starr, B.S., 1995. Do NMDA receptor-mediated changes in motor behavior involve nitric oxide? Eur. J. Pharmacol. 272, 211–217.

Steiner, A.A., Carnio, E.C., Autunes-Rodrigues, J., Branco, L.G., 1998. Role of nitric oxide in systemic vasopressin-induced hypothermia. Am. J. Physiol. 275, R937–R941.

Verrecchia, C., Buisson, A., Lakhmeche, N., Plotkine, M., Boulu, R.G., 1994. Nitric oxide and cerebral ischemia. Ann. N.Y. Acad. Sci. 738, 341–347.

Vincent, S.R., Kimura, H., 1992. Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience 46, 755–784.

Volke, V., Koks, S., Vasar, E., Bourin, M., Bradwejn, J., Mannisto, P.T., 1995. Inhibition of nitric oxide synthase caused anxiolytic-like behavior in an elevated plus-maze. NeuroReport 6, 1413–1416.

Volke, V., Soosaar, A., Koks, S., Bourin, M., Mannisto, P.T., Vasar, E., 1997. 7-Nitroindazole, a nitric oxide synthase inhibitor, has anxiolytic-like properties in exploratory models of anxiety. Psychopharmacology 131, 399–405.

Winslow, J.T., Insel, T.R., 1990. Serotonergic and catecholaminergic reuptake inhibitors have opposite effects on the ultrasonic isolation calls of rat pups. Neuropsychopharmacology 3, 51–59.

Winslow, J.T., Insel, T.R., 1991. The infant rat separation paradigm: a novel test for novel anxiolytics. Trends Pharmacol. Sci. 12, 402–404.

Winslow, J.T., Insel, T.R., Trullas, R., Skolnick, P., 1990. Rat pup isolation calls are reduced by functional antagonists of the NMDA receptor complex. Eur. J. Pharmacol. 190, 11–21.

Zhang, J., Snyder, S.H., 1995. Nitric oxide in the nervous system. Annu. Rev. Pharmacol. Toxicol. 35, 213–233.