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Nitric oxide modulates retention of immobility in the forced swimming test in rats

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Abstract

Although originally developed as a possible screen for antidepressants, the Porsolt forced swimming test has more recently been extensively used as a model for studying the involvement of the endocrine system in the acquisition and retention of behavioural responses. In previous studies we have shown that while adrenalectomised rats acquire the immobile response normally, they are unable to retain it on retest next day. In the present study we show that retention of the immobile response in the Porsolt swim test is impaired in intact rats given the nitric oxide (NO) inhibitor L-*N*-arginine methyl ester (L-NAME), in a dose- and time-dependent manner. At a dose of 50 mg/kg levels of immobility are similar to those in adrenalectomised animals, an effect reversed by the simultaneous administration of L-arginine (50 mg/kg). L-Arginine also reverses the behavioural effect of adrenalectomy, and L-NAME blocks the ability of dexamethasone or the κ-selective opioid ketocyclazocine to reverse the effect of adrenalectomy on retention of the immobile response. We conclude that the κ-opioid and glucocorticoid mediated pathways previously shown to independently facilitate retention are mediated by nitric oxide.

Keywords: L-*N*-Arginine methyl ester (L-NAME); Nitric oxide (NO); Forced swimming test; Adrenalectomy; Glucocorticoid

1. Introduction

Porsolt et al. (1977, 1978)) developed a forced swimming test as a screen for potential anti-depressant drugs, and subsequently we and others have investigated the endocrine modulation of the acquisition and retention of the immobile (floating) response shown by rats in this model. Naive rats placed in a Plexiglas cylinder initially swim actively, but with increasing periods of immobility, so that during the last 5 min of a 15 min test period they are immobile for ~70% of the time. On a 5 min retest next day, intact rats are similarly immobile for ~70% of the time, evidence that they have retained the immobile response acquired the day before.

Rats adrenalectomised 4–6 days prior to an initial 15 min swimming test show progressive levels of immobility indistinguishable from those in intact rats. On retest 24 h later, however, adrenalectomised rats do not retain the acquired response, with markedly reduced levels of immobility (~30%) compared with intact controls (~70%). This effect is equally reversed by either glucocorticoids (Jefferys et al., 1983; Veldhuis et al., 1985) or κ-selective opioids (Jefferys et al., 1985) administered before test, but not just before retest.

Administration of high doses (10 mg/kg) of the μ-preferring opiate receptor antagonist naloxone not only blocked the effect of administered ketocyclazocine, but also of dexamethasone; in addition, similar doses of naloxone to intact rats mimicked the effects of adrenalectomy (Jefferys et al., 1984). These findings led to the hypothesis that retention of the immobile response may be mediated by glucocorticoid or opioid pathways acting via a final common opioidergic effector. That the two pathways independently facilitate retention was shown by studies in which the anti-glucocorticoid RU38486 and the κ-selective antagonist MR2266 were administered alone or together. Given alone, both were without effect on retention, but when simultaneously administered levels of immobility were similar to those in adrenalectomised rats (Jefferys and Funder, 1987).

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It has previously been shown that nitric oxide can modulate neuronal function in general (Schuman and Madison, 1994) and the acquisition and retention of learned behaviours in particular (Bohme et al., 1993; Chapman et al., 1992; Hölscher and Rose, 1992). In the present study we have thus investigated the effects of blocking nitric oxide synthesis with L-NAME, a nitric oxide synthesis inhibitor, on the retention of the immobile floating response in intact rats. We have also determined the effect of L-arginine on the behavioural effect of adrenalectomy, and the effect of L-NAME on the glucocorticoid and κ-opioid reversal of the effects of adrenalectomy, to explore whether nitric oxide mediates retention of the immobile floating response seen at retest in both intact rats and adrenalectomised rats on replacement therapy, and thus fulfils the criteria for a final common effector in procuring the behavioural response.

2. Materials and methods

Male Sprague-Dawley rats weighing 160–180 g from the Central Animal House Monash University, Melbourne, Australia were maintained on a 12 : 12 light/dark cycle with lights on at 06.00 h. The test procedure described by Porsolt et al. (1977, 1978) was closely followed with rats placed in cylinders (diameter 18 cm, height 40 cm) for a 15 min test period followed 24 h later by a 5 min retest, with the absence of hind leg movement recorded as immobility by stop watch cumulation by a constant observer during both exposures. Both testing and retesting were conducted between 06.00–08.00 h. Rats were adrenalectomised via the dorsal route under ether anaesthesia and maintained post-operatively on 0.9% NaCl for 4–6 days prior to use. All drugs were given 15 min before the initial exposure unless otherwise stated. L-Arginine and *N*-L-arginine methyl ester were from Sigma (St. Louis, MO, USA) and were prepared in saline and administered in 100 μl solution (L-arginine) or suspension (L-NAME). Ketocyclazocine was from Sterling Winthrop (gift of Prof. A.L.A. Boura, Newcastle University) and was given at a dose of 7.5 μg/rat. Dexamethasone Na^+ phosphate was from Merck, Sharp and Dohme, Sydney, Australia and was administered at a dose of 6 μg/rat. Data were compared by analysis of variance and Dunnett's *T* post-hoc analysis (Winer, 1970). All animal procedures were approved by the Animal Ethics Committee of the Baker Medical Research Institute.

3. Results

The effect of the nitric oxide inhibitor L-NAME on the retention of the immobile response in intact animals is shown in Fig. 1. At the two lower doses (5, 25 mg/kg) no difference was noted, but at 50 mg/kg animals were immobile for 41% of the retest period, a level similar to those previously reported for adrenalectomised animals on retest (Jefferys et al., 1983). L-NAME had no effect on acquisition at any dose (data not shown). That the effect of L-NAME can be opposed by the simultaneous administration of an equal dose of L-arginine is shown in Fig. 2. Intact rats given L-NAME alone were immobile for 44% of the retest period; when L-arginine was simultaneously administered immobility was restored to levels (77%) indistinguishable from control (76%).

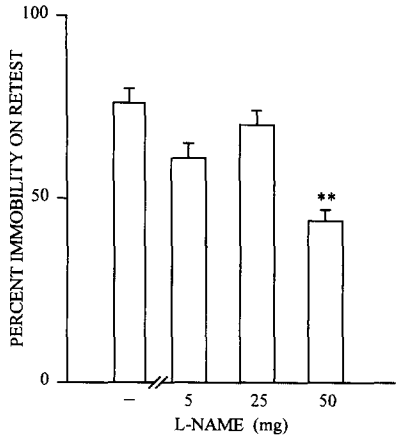

Fig. 1. The effect of the nitric oxide synthesis blocker L-NAME administered at different doses (5–50 mg/kg) on levels of immobility during the 5 min retest 24 h following the initial 15 min swimming exposure. Values shown are mean ± S.E.M., $n = 8$. $F(3,28) = 6.41$. ** $P < 0.01$ vs. control.

Fig. 3 shows the effect of L-NAME (50 mg/mg), given to intact rats at intervals from 30 min to 23 h post-test, on levels of retention at retest. A significant reduction in immobility was seen only when L-NAME

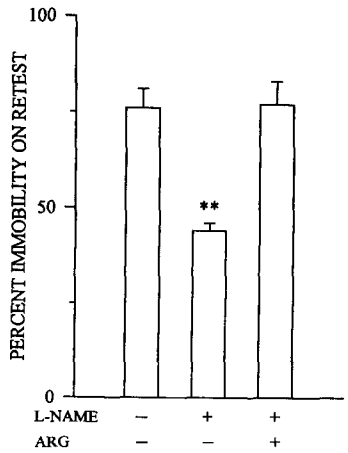

Fig. 2. The effect of L-arginine (50 mg/kg) on the behavioural effect of L-NAME (50 mg/kg) on retention of the immobile response in intact rats. $F(2,21) = 14.7$. ** $P < 0.01$ vs. other groups.

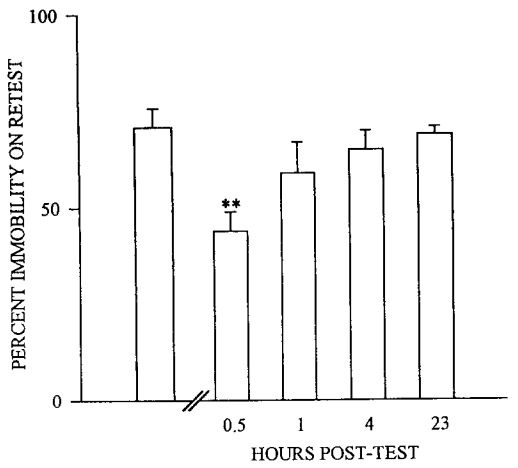

Fig. 3. Percent immobility during the 5 min retest shown by intact rats administered L-NAME (50 mg/kg) between 30 min and 23 h after the initial swimming exposure. Mean ± S.E.M. values are shown. $n = 8$. $F(4,35) = 44.5$. ** $P < 0.01$ vs. control.

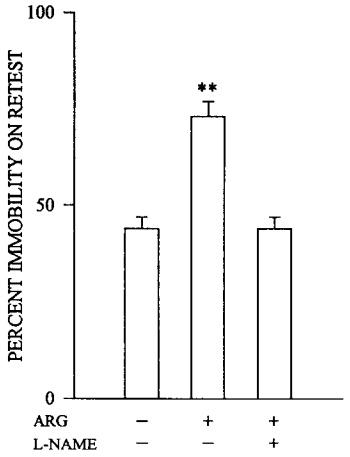

Fig. 5. The effect of L-NAME (50 mg/kg) on the reversal of the behavioural effect of adrenalectomy by L-arginine (50 mg/kg) during the 5 min retest. Mean ± S.E.M. values are shown. $n = 8$. $F(2,21) = 30.1$. ** $P < 0.01$ vs. other groups.

was administered at 30 min post-test, with animals immobile for 44% of the retest period. Fig. 4 shows the effect of L-arginine given to adrenalectomised rats. At the lowest dose (5 mg/kg) L-arginine was without effect, with the levels of immobility (45%) similar to adrenalectomised (43%). At the intermediate dose (25 mg/kg) levels of immobility (54%) were significantly higher than in adrenalectomy, and significantly lower than in intact animals. The highest dose (50 mg/kg) clearly reverses the behavioural effect of adrenalectomy with animals immobile for 77% of the retest period, a value similar to that in intact controls and in adrenalectomised rats administered glucocorticoids or κ-selective opioids (Jefferys et al., 1983, 1985).

Fig. 5 shows that when adrenalectomised animals are simultaneously given L-arginine 50 mg/kg and L-NAME 50 mg/kg the restorative effect of L-arginine is itself reversed, with the rats immobile for 44% of the retest period, a level of immobility similar to vehicle-treated adrenalectomised rats. Fig. 6 shows that, as previously described, ketocyclazocine and dexamethasone reverse the effect of adrenalectomy, with animals immobile for 76% (ketocyclazocine) and 70% (dexamethasone) of the retest period. When L-NAME 50 mg/kg was simultaneously administered with either ketocyclazocine or dexamethasone these behavioural effects were blocked, with rats immobile for 43% (ketocyclazocine) or 39% (dexamethasone) of the retest period.

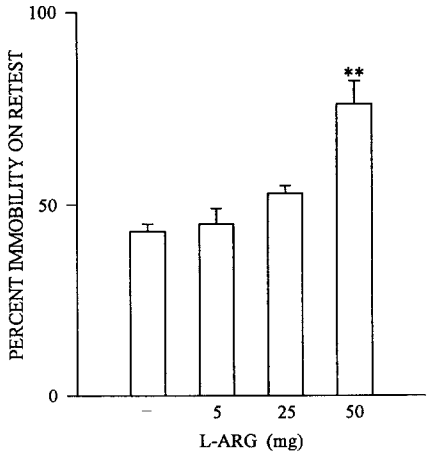

Fig. 4. The effect of different doses of L-arginine (5–50 mg/kg) administered to rats 4–6 days post-adrenalectomy on levels of immobility during the 5 min retest. Mean ± S.E.M. values are shown. $n = 8$. $F(3,28) = 16.0$. ** $P < 0.01$ vs. control.

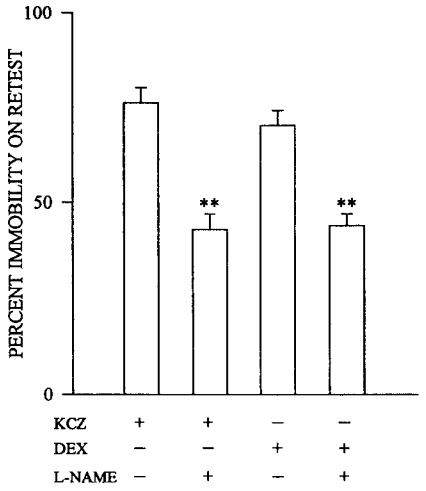

Fig. 6. The effect of L-NAME on the ketocyclazocine and dexamethasone reversal of the behavioural effect of adrenalectomy on levels of immobility during the 5 min retest. Ketocyclazocine (7.5 μg/rat) and dexamethasone (6 μg/rat) were administered alone or simultaneously with L-NAME (50 mg/kg). Values shown are mean ± S.E.M. $F(3,28) = 35.3$. ** $P < 0.01$ vs. groups with L-NAME.

4. Discussion

In our previous studies we have shown that adrenalectomised animals swum in the Porsolt test had no difficulty acquiring the immobile response, but that their retention of this response as impaired, in that their levels of immobility on retest were only ~ 30% of the 5 min period compared with controls (~ 70%). This behavioural effect of adrenalectomy was reversed by either glucocorticoids or κ-selective opioids, with the effects of both able to be blocked by naloxone; in addition, high-dose naloxone given to intact rats was shown to mimic the effects of adrenalectomy (Jefferys et al., 1983, 1985). These findings led to the hypothesis of two independent (glucocorticoid, κ-opioid) pathways subserving incorporation and retention of the behavioural response, with a final common opioidergic effector pathway. An alternative explanation for the observed inhibitory effects of naloxone is that the very high doses used had non-specific behavioural effects, consistent for example with the described anorexigenic action of antagonist administration at such levels (Kastin et al., 1984).

In the present studies, the finding that L-NAME was without effect on the acquisition of the immobile response in intact and adrenalectomised animals suggests that nitric oxide action is not involved in acquisition. This finding is similar to that of Hölscher and Rose (1992), who showed that blocking nitric oxide generation is without effect in the acquisition of a passive avoidance task. In contrast, blocking nitric oxide generation impairs or abolishes acquisition of a partial learning task and classical conditioned responses (Bohme et al., 1993; Chapman et al., 1992), so that nitric oxide generation appears to be required for the acquisition of some learned behaviours but not others.

The present studies also clearly show that nitric oxide modulates retention of the immobile response at retest, in that intact rats administered L-NAME showed significantly impaired levels of immobility, with this behavioural effect blocked by L-arginine simultaneously administered with L-NAME. In addition, this effect of L-NAME was clearly dose- and time-dependent, with retention impaired only when the blocker was administered within 30 min of the initial swimming exposure. In our previous studies activation of glucocorticoid and κ-selective pathways up to 1 h post-test facilitated retention (Jefferys et al., 1983; Jefferys and Funder, 1987); whether or not the apparently more time-limited effect of nitric oxide in modulating retention reflects between experiment variation, or a real difference in half-life of the processes involved in the proposed effector cascade, remains to be explored.

Further evidence for the crucial role of nitric oxide in such an effector cascade is shown by the effect of L-arginine in adrenalectomised animals. L-Arginine clearly reversed the behavioural effect of adrenalectomy; that this reversal was specific was shown by the simultaneous administration of L-NAME, which in turn blocked the effect of arginine. The effect of ketocyclazocine or dexamethasone given to adrenalectomised rats to reverse the behavioural effect of adrenalectomy was similarly blocked by L-NAME, further evidence for an nitric oxide dependent step distal to the pathways activated by adrenal signals from cortex or medulla.

Previous studies have demonstrated the importance of the dentate gyrus in the hippocampus in the glucocorticoid effects on retention (De Kloet et al., 1988). In the central nervous system nitric oxide is generated postsynaptically but has its effect presynaptically, following release into the extracellular space (Bredt and Snyder, 1992). Histochemical studies have shown the presence of nitric oxide in the hippocampus (Vincent and Kimura, 1992); that hippocampal nitric oxide dependent pathways may be involved in acquisition and retention is consistent with the finding that long term potential in hippocampal slices is inhibited by L-*N*-arginine, a nitric oxide inhibitor (Bohme et al., 1993). The present findings show that nitric oxide has a crucial role in facilitating retention of the immobile response at retest, and that nitric oxide mediates the effects of activation of glucocorticoid and κ-opioid pathways previously identified as facilitating retention.

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