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Swim-stress-induced antinociception in young rats

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1 Opioid and non-opioid mechanisms have been implicated in the phenomenon of stress-induced antinociception in adult rodents. We have studied stress-induced antinociception in developing rats and characterized differences in the neurochemical basis of this effect in pre- and post-weanling animals.

2 Twenty and 25 day old rats were stressed using warm water (20°C) swimming for 3 or 10 min periods and antinociception was assessed by the tail immersion test (50°C).

3 A 3 min swim in 20 and 25 day old rats produced marked antinociception which was blocked by naloxone, Mr 1452, 16-methyl cyprenorphine and levallorphan but not Mr 1453 or N-methyl levallorphan. The δ-opioid receptor antagonist ICI 174,864 attenuated stress-induced antinociception in 25 day old rats but was without effect in 20 day old animals.

4 A 10 min swim in 25 day old rats produced antinociception which was non-opioid in nature. In contrast, antinociception was not observed in 20 day old rats after a 10 min swim-stress.

5 Pretreatment of animals with dexamethasone blocked 3 min swim-stress antinociception in 20 and 25 day old animals but had no effect on antinociception induced by a 10 min swim.

6 Swim-stress-induced antinociception can be observed in young rats and dissociated into opioid and non-opioid types dependent on the duration of swimming stress. The non-opioid type appears to develop more slowly and cannot be observed in preweanling rats. The opioid type is a predominantly μ-receptor phenomenon in preweanling animals but δ-receptor components are observable in postweanling rats.

Introduction

There is a substantial literature which demonstrates the involvement of endogenous opioid peptides in the control of stress in both experimental animals and in man. One phenomenon which has been extensively studied in adult rodents is that of stress-induced antinociception (SIA). For example, stressors such as footshock (Madden *et al.*, 1977), swimming (Bodnar *et al.*, 1978a), 2-deoxy-D-glucose treatment (Bodnar *et al.*, 1978b), food deprivation (McGivern *et al.*, 1979), and immobilisation (Amir & Amit, 1978) have all been shown to produce antinociception in the adult rat. The receptor systems involved in SIA are stressor-dependent and opioid and/or non-opioid mechanisms have been implicated (see Bodnar, 1984). There are relatively few studies of SIA in developing animals but these indicate that this effect can be observed in the neonate (Bardo *et al.*, 1981; Hamm & Knisely, 1984; 1987; Kehoe & Blass, 1986). We describe here studies on SIA in 20 and 25 day old rats using warm water swimming as the stress procedure, and demonstrate that the nature of this response is markedly different at these postnatal ages.

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Methods

Animals and experimental conditions

Wistar albino rats (University of Surrey strain) of either sex were used in all experiments and maintained at a constant temperature (21 ± 1°C) and light/dark cycle (lights on at 07 h 00 min). Litters were culled to 8 pups per dam at birth and where possible cross-fostered. Neonates were weaned at

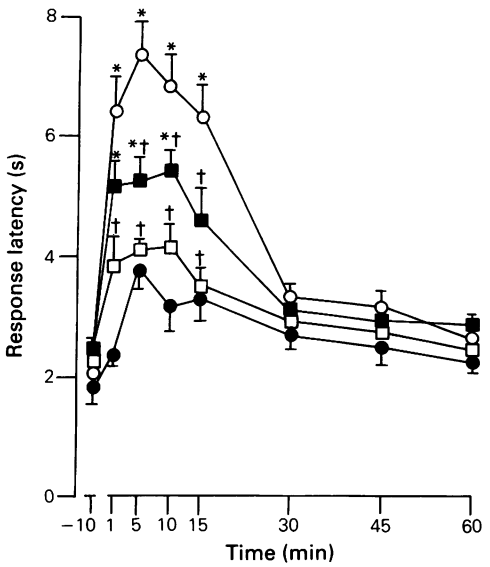

Figure 1 Effect of naloxone on antinociception induced by a 3 min swim in 20 day old rats. Values represent means and vertical lines s.e.mean for groups of 10 animals. (●) Saline injected, unstressed; (○) saline injected, swim-stressed; (■) naloxone ($1\,mg\,kg^{-1}$), swim-stressed; (□) naloxone ($10\,mg\,kg^{-1}$), swim-stressed. Significant differences from non-stressed controls are indicated by $*P < 0.05$ and from swim-stressed controls by $\dagger P < 0.05$.

day 21. All experimental procedures were carried out in a quiet, windowless, air-conditioned laboratory and animals equilibrated to these conditions for 2 h before experimentation. Twenty day old pups remained with the mother, except during drug administration, swimming and antinociceptive testing, to minimize stress due to maternal deprivation. Each series of determinations represents measurements made on five separate litters on at least two separate days to avoid inter-litter and inter-day variation. All procedures were carried out between 13 h 00 min and 17 h 00 min.

Swim-stress procedures and antinociceptive testing

Each litter was divided into four treatment groups. One group received 0.9% saline i.p. and remained in the home cage as a control group. The other 3 groups were injected i.p. with saline or appropriate drug (using a blind procedure and a dose volume of $0.1\,ml\,20\,g^{-1}$) and 10 min later placed individually in 20°C water for 3 or 10 min. The water was contained in 2 litre beakers (14 cm in diameter; 12 or 20 cm deep for 20 and 25 day old pups, respectively). At the end of the swimming period pups were gently dried before antinociceptive testing. For studies with dexamethasone, rats were injected 24 h ($0.4\,mg\,kg^{-1}$) and 1 h ($0.2\,mg\,kg^{-1}$) before swimming as described by Lewis *et al.* (1980). Rectal temperatures were measured before drug administration and after swimming stress using an Edale temperature probe.

Antinociception was assessed by the tail immersion test (50°C) as described by Kitchen *et al.* (1984) for neonatal rats. Nociceptive responses were measured immediately before drug administration and 1, 5, 10, 15, 30, 45 and 60 min following the swim-stress. Treatment group means were statistically compared using analysis of variance and Dunnett's test.

Drugs

Dexamethasone was purchased from Sigma Chemical Co., U.K. The following were gifts: naloxone hydrochloride (Du Pont Pharmaceuticals); 16-methyl cyprenorphine (M8008; Reckitt & Colman); Mr 1452/3 ((+)/(−)-5,9-α-dimethyl-2-(3-furylmethyl) -2'-hydroxy-6,7-benzomorphan methane sulphonate; Boehringer Ingelheim); levallorphan (Roche Products); N-methyl levallorphan (Sanofi Research); ICI 174,864 (N,N-bisallyl-Tyr-Aib-Aib-Phe-Leu-OH, Aib = α-amino-isobutyric acid; I.C.I. Pharmaceuticals). All drugs were dissolved in saline with the exception of ICI 174,864 which was suspended in saline and diluted with an equivalent volume of 0.1 M L-arginine (Sigma) and 16-methyl cyprenorphine which was dissolved in 0.1 M HCl and neutralised with $NaHCO_3$.

Results

Nociceptive latencies in 20 and 25 day old rats not subjected to swimming stress showed small but significant increases after intraperitoneal injection of saline (Figures 1–6). This effect was most marked 15 min after injection and thereafter nociceptive latencies fell to pre-injection values. The doses of the opioid antagonists levallorphan, N-methyl levallorphan, naloxone, Mr 1452/3, 16-methyl cyprenorphine and ICI 174,864 used in the current study had no effect on nociceptive latencies in unstressed 20 or 25 day old rats (data not shown). In addition, no overt behavioural effects were observed for any of these antagonists.

A 3 min swim at 20°C in both 20 and 25 day old rats produced marked antinociception which became evident 1 min after the swimming period and persisted for at least 15 min. At both ages this effect was significantly attenuated by naloxone (1 and $10\,mg\,kg^{-1}$) (Figures 1 and 2). In 20 day old rats antinociception induced by a 3 min swimming period

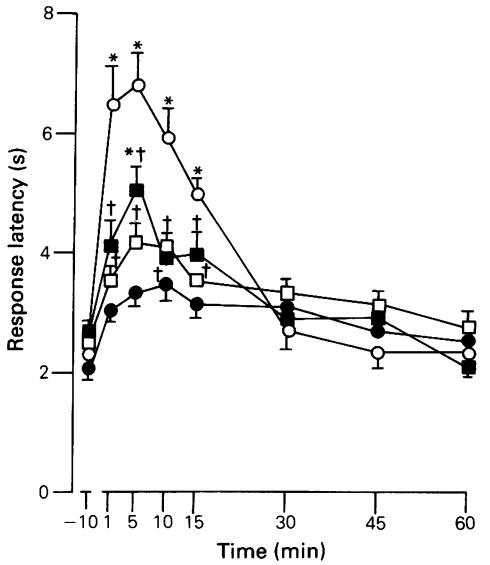

Figure 2 Effect of naloxone on antinociception induced by a 3 min swim in 25 day old rats. Values represent means and vertical bars s.e.mean for groups of 10 animals. (●) Saline injected, unstressed; (○) saline injected, swim-stressed; (■) naloxone ($1\,mg\,kg^{-1}$), swim-stressed; (□) naloxone ($10\,mg\,kg^{-1}$), swim-stressed. Significant differences from non-stressed controls are indicated by $*P < 0.05$ and from swim-stressed controls by $\dagger P < 0.05$.

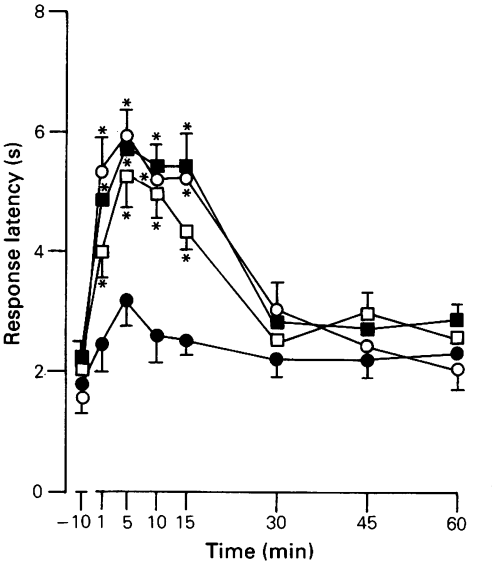

Figure 3 Effect of ICI 174,864 on antinociception induced by a 3 min swim in 20 day old rats. Values represent means and vertical lines s.e.mean for groups of 10 animals. (●) Vehicle injected, unstressed; (○) vehicle injected, swim-stressed; (■) ICI 174,864 ($1\,mg\,kg^{-1}$), swim-stressed; (□) ICI 174,864 ($10\,mg\,kg^{-1}$), swim-stressed. Significant differences from non-stressed controls are shown by $*P < 0.05$.

Table 1 Effects of various opioid antagonists on peak antinociceptive effects of 3 min swimming stress in 20 day old rats

Drug treatment	*Tail immersion latencies* (s)
Vehicle control	5.8 ± 0.3
Levallorphan ($10\,mg\,kg^{-1}$)	3.4 ± 0.3*
N-methyl levallorphan ($10\,mg\,kg^{-1}$)	5.3 ± 0.3
Vehicle control	6.5 ± 0.5
Mr 1452 ($10\,mg\,kg^{-1}$)	3.2 ± 0.3*
Mr 1453 ($10\,mg\,kg^{-1}$)	5.6 ± 0.4
Vehicle control	5.5 ± 0.3
16-methyl cyprenorphine ($1\,mg\,kg^{-1}$)	3.5 ± 0.3*
16-methyl cyprenorphine ($10\,mg\,kg^{-1}$)	3.0 ± 0.2*

Results are expressed as mean values ± s.e.mean for groups of 10 animals. Peak effects represent measurements 5 min after swimming stress. The time course profile of antagonism, where observed, was similar to that shown for naloxone (Figure 1). Significant differences from swim-stressed controls are denoted by $*P < 0.05$.

was blocked by Mr 1452 ($10\,mg\,kg^{-1}$) but not its stereoisomer Mr 1453 (Table 1). In addition, 16-methyl cyprenorphine (1 and $10\,mg\,kg^{-1}$) and levallorphan ($10\,mg\,kg^{-1}$) blocked swim-stress-induced antinociception in 20 day old rats but a $10\,mg\,kg^{-1}$ dose of the quaternary derivative N-methyl levallorphan was ineffective (Table 1). The δ-receptor antagonist ICI 174,864 (1 and $10\,mg\,kg^{-1}$) significantly reduced 3 min swim-stress antinociception in 25-day old rats but exhibited no antagonism in 20 day old animals (Figures 3 and 4).

Twenty day old rats subjected to a 10 min swimming period showed no increase in nociceptive latencies up to 1 h after swimming (Figure 5). In contrast, 25 day old rats exhibited antinociception after a 10 min swim, an effect not antagonized by up to $10\,mg\,kg^{-1}$ naloxone (Figure 6) or Mr 1452 (data not shown).

Pretreatment of animals with dexamethasone blocked 3 min swim-stress antinociception in 20 and 25 day old animals but was without effect upon antinociception induced by a 10 min swimming period in 25 day old rats (Table 2). Dexamethasone treatment *per se* was without effect on nociceptive latencies.

Marked hypothermia was observed immediately after swimming stress in both 20 day (1 min post

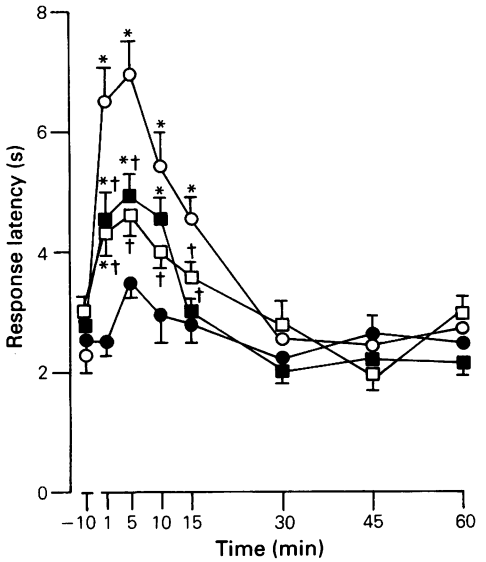

Figure 4 Effect of ICI 174,864 on antinociception induced by a 3 min swim in 25 day old rats. Values represent means and vertical lines s.e.mean for groups of 10 animals. (●) Vehicle injected, unstressed; (○) vehicle injected, swim-stressed; (■) ICI 174,864 (1 mg kg^{-1}), swim-stressed; (□) ICI 174,864 (10 mg kg^{-1}), swim-stressed. Significant differences from non-stressed controls are indicated by * $P < 0.05$ and from swim-stressed controls by † $P < 0.05$.

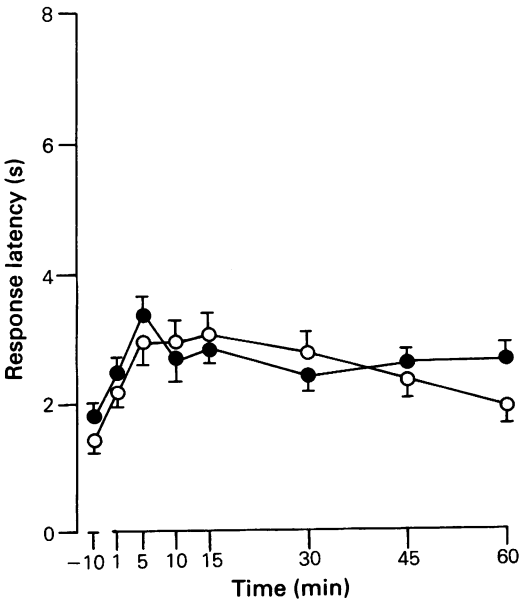

Figure 5 Effect of a 10 min swim on tail immersion latencies in 20 day old rats. Values represent means and vertical lines s.e.mean for groups of 10 animals. (●) Saline injected, unstressed; (○) saline injected, swim-stressed.

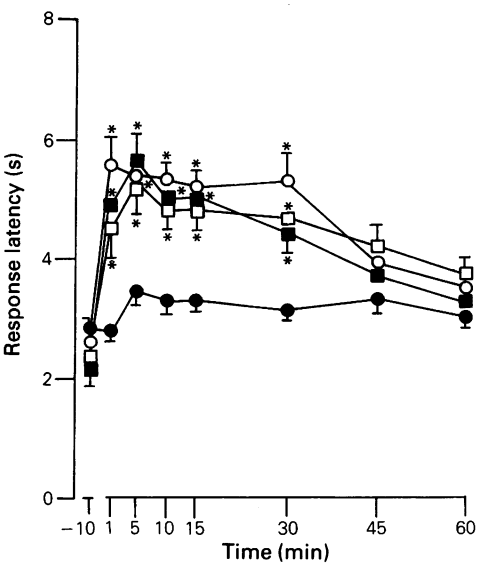

Figure 6 Effect of naloxone on antinociception induced by a 10 min swim in 25 day old rats. Values represent means and vertical lines s.e.mean for groups of 10 animals. (●) Saline injected, unstressed; (○) saline injected, swim-stressed; (■) naloxone (1 mg kg^{-1}), swim-stressed; (□) naloxone (10 mg kg^{-1}), swim-stressed. Significant differences from non-stressed controls are shown by * $P < 0.05$.

swim: 36.8 ± 0.2 vs 28.2 ± 0.2°C (3 min); 37.1 ± 0.1 vs 23.7 ± 0.2°C (10 min)) and 25 day old rats (1 min post swim: 37.8 ± 0.2 vs 31.5 ± 0.9°C (3 min); 37.8 ± 0.1 vs 24.6 ± 0.6°C (10 min)). This response,

Table 2 Effect of dexamethasone treatment on peak antinociceptive effects of 3 and 10 min swimming stress in 20 and 25 day old rats

	Tail immersion latencies (s)	
Age/stress	*Control*	*Dexamethasone treated*
20d/unstressed	2.9 ± 0.2	3.0 ± 0.3
20d/3 min swim	5.5 ± 0.3*	3.8 ± 0.2*†
25d/unstressed	3.4 ± 0.3	3.1 ± 0.4
25d/3 min swim	6.7 ± 0.5*	4.2 ± 0.3†
25d/unstressed	3.6 ± 0.2	3.3 ± 0.2
25d/10 min swim	5.5 ± 0.2*	5.3 ± 0.2*

Results are expressed as mean values ± s.e.mean for groups of 10 animals. Peak effects represent measurements 5 min after swimming stress. The time course profile of antagonism, where observed, was similar to that shown for naloxone (Figures 1 and 2). Significant differences from non-stressed controls are indicated by * $P < 0.05$ and from swim-stressed controls by † $P < 0.05$.

which was not accompanied by pronounced behavioural depression, persisted for 30 and 45 min respectively for 3 and 10 min swims in 20 day old animals but for 25 day old rats the duration of hypothermia was shorter (15 and 30 min respectively for 3 and 10 min swims). Naloxone at 1 and 10 mg kg^{-1} did not significantly alter the hypothermic effects of swimming (data not shown).

Discussion

Short warm water swims in our study produced SIA in 20 and 25 day old rats confirming other ontogenetic studies using maternal deprivation (Kehoe & Blass, 1986), electric shock (Hamm & Knisely, 1984) and cold water swimming (Hamm & Knisely, 1987) as stressors. The temperature and duration of swimming has been shown in adult rats to alter markedly the neurochemical basis of the SIA. In the adult, short swimming periods in warm water are sensitive to naltrexone indicating opioid receptor mediated antinociception, whereas longer or cold water swims are insensitive to opioid antagonists (Terman *et al.*, 1986). The reversal of short warm water swim SIA in 20 and 25 day old rats by the opioid antagonists naloxone and Mr 1452 but not Mr 1453 demonstrates that this phenomenon, as in the adult, is opioid receptor-mediated and also that it exhibits stereoselectivity. The relatively high concentration of naloxone required to antagonize completely this SIA might suggest the involvement of non-μ opioid receptor subtypes. It is, however, unlikely that κ- or δ-sites are involved in SIA in 20 day old rats since 16-methyl cyprenorphine which has a low affinity for κ-receptors (Smith, 1987) completely blocks this response and ICI 174,864, a selective δ-antagonist (Cotton *et al.*, 1984) is without effect in this age group. However, it should be pointed out that there do appear to be neurochemical dissimilarities in the SIA produced by short swims at 20 and 25 days of age, since the latter is antagonized by the δ-antagonist ICI 174,864. This suggests that δ-receptors may be important in this phenomenon at later postnatal ages and accords with the delayed development of this receptor subtype (McDowell & Kitchen, 1986) and with the work of others who have shown antagonism of SIA by ICI 174,864 in mice (Hart *et al.*, 1985). Nevertheless most lines of evidence, including antagonism of SIA by levallorphan, suggest that μ-sites may play a role in this antinociceptive response. Moreover, the lack of effect of the quaternary derivative N-methyl levallorphan which does not pass the blood brain barrier and which acts as a peripheral opioid antagonist *in vivo* (Dragonetti *et al.*, 1983; personal observations), confirms the central nature of swimming SIA in young rats. This contrasts with results in the adult showing that quaternary naloxone significantly reduces continuous footshock stress indicating a peripheral component (Chance & Nelson, 1986).

The possibility that the observed SIA does not truly reflect a reduced pain perception and is secondary to alterations in, for example, thermoregulation, was assessed by monitoring rectal temperature. Like others we were able to dissociate the hypothermic and antinociceptive effects of swim stress (Bodnar *et al.*, 1978c; Terman *et al.*, 1986) and, moreover, the hypothermia was unaffected by naloxone. It therefore appears that true SIA can be observed in young rats.

As in adult rats, a naloxone-resistant SIA can be observed by longer swimming in 25 day old rats, effects which have also been observed with increasing stress severity using footshock (Terman *et al.*, 1984). As in adults, this antinociception is not antagonized by dexamethasone, in contrast to the shorter swimming SIA, suggesting involvement of the hypothalamus-pituitary-adrenal axis only in the opioid-mediated SIA (Lewis *et al.*, 1980). We were unable to observe antinociception in 20 day old animals subjected to a 10 min swim, suggesting that the neurochemical basis for non-opioid SIA does not develop until after the third postnatal week.

None of the opioid antagonists *per se* produced hyperalgesia in these young animals, an effect which has been obtained for naloxone in some studies in adult rats (Jacob & Ramabadran, 1977). In addition there was no indication of any μ-agonist activity which has been observed with the δ-antagonist ICI 174,864 and its metabolite product in isolated tissue studies (Cohen *et al.*, 1986).

In conclusion, swim SIA can be observed in 20 day and 25 day old rats. Short swims produce opioid-mediated antinociception, which appears to be predominantly a μ-receptor phenomenon but shows a δ-receptor component at the later age. Longer swims produce a non-opioid antinociception only at the older age suggesting that non-opioid pain modulatory systems develop more slowly than those dependent on endogenous opioids.

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