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# α-Adrenoceptor Involvement in Swim Stress-induced Antinociception in the Mouse

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**Abstract**—Three different intensities of swim stress produced stress-induced antinociception (SIA) in mice which was assessed either by the reduction in the number of abdominal constrictions produced by acetic acid or by an increase in reaction time on a hot-plate. The involvement of α-adrenoceptors in the three models of SIA was investigated using selective antagonists. SIA produced by the mild stress of a 30 s warm water swim was attenuated by idazoxan (0·5–1 mg $kg^{-1}$), and by yohimbine at a dose (1 mg $kg^{-1}$) which reduced antinociception produced by clonidine (12·5–50 μg $kg^{-1}$). Indoramin (1–2 mg $kg^{-1}$) did not affect this model of SIA, but reversed phenylephrine induced inhibition of the constrictions. A 3 min room temperature swim increased reaction times on the hot-plate and this naloxone-sensitive SIA was reduced significantly by prazosin (1–2 mg $kg^{-1}$), idazoxan (0·5–1 mg $kg^{-1}$) and yohimbine (0·5–1 mg $kg^{-1}$) but enhanced by clonidine (0·5 mg $kg^{-1}$) and noradrenaline (NA) (10 μg i.c.v.). Mice treated with 6-hydroxydopamine (60 + 60 μg i.c.v.) were hypersensitive to the hot-plate and did not develop SIA. Levels of noradrenaline in the brain (minus the cerebellum) were decreased after the room temperature swim SIA. The most severe stress of a cold water swim produced SIA on the hot-plate which was initially naloxone-insensitive. Both components of the cold water swim SIA were enhanced by NA (10 μg i.c.v.) and reduced by yohimbine (5 μg i.c.v.) whilst prazosin (5 μg i.c.v.) inhibited only the later naloxone-sensitive component; and these adrenoceptor agents potentiated cold water swim hypothermia induced by the cold water swim. These results show the differential involvement of $\alpha_1$- and $\alpha_2$-adrenoceptors in SIA in the mouse.

Stress-induced antinociception (SIA) is a complex phenomenon. Many noxious, non-noxious and environmental stimuli may induce changes in pain sensitivity. Most commonly, a rise in nociceptive threshold, i.e. antinociception, has been described in rodents following administration of electric foot-shocks (Hayes et al 1978; Terman et al 1984; Curzon et al 1986), immobilization (Amir & Amit 1978) and warm and cold water swims (Bodnar et al 1978; Hart et al 1983; Hart & Oluyomi 1986, 1989). Although several neurotransmitters have been implicated, full details of the neuronal pathways involved have not yet been elucidated (Curzon et al 1986). This is largely because the characteristics of a particular model of SIA depend not only on the animal species but the type and strength of the stress and the intensity of noxious stimuli used to assess the antinociception. Both opioid and non-opioid forms of SIA have been described (Bodnar et al 1978; Hart et al 1983) and the involvement of other neurotransmitters such as noradrenaline and 5-hydroxytryptamine has been suggested (Snow et al 1982).

Noradrenaline (NA) has been shown to attenuate spinal nociceptive transmission. Iontophoretic application of NA depressed dorsal horn nociceptive neurons while intrathecal NA produced dose-dependent antinociception which was mediated by α-adrenoceptors (Reddy et al 1980). Clonidine has been shown to be antinociceptive in the rat (Paalzow & Paalzow 1976; Fielding et al 1978) and cat (Reddy et al 1980) or after direct injection into the nucleus raphe magnus (NRM) (Sagen & Proudfit 1982). Further evidence for the involvement of $\alpha_2$-adrenoceptors was obtained by Reddy et al (1980) who reduced NA-induced antinociception with yohimbine and by Sagen & Proudfit (1982) who observed hyperalgesia after the direct application of yohimbine to the NRM in the rat. Confirmation of the importance of $\alpha_2$-adrenoceptors in the rat was obtained by Hayes et al (1986a) who observed antinociception in the paw pressure test with the more selective agonists, guanabenz and guanfacine. However, experiments with the selective antagonists WB4101 ($\alpha_1$) and idazoxan ($\alpha_2$) demonstrated that antinociception in the rat may be mediated by both $\alpha_1$- and $\alpha_2$-adrenoceptors (Hayes et al 1986b).

Investigations of the involvement of noradrenergic neurons in SIA have produced contradictory results. SIA in rats after a cold water swim (CWS) is potentiated by both clonidine (Bodnar et al 1983) and yohimbine (Kepler & Bodnar 1988), whilst SIA occurring as a result of foot-shock was reduced by both clonidine (Snow et al 1982) and yohimbine (Coderre & Rollman 1984). Jensen & Smith (1982) have concluded that neither blockade of adrenoceptors nor inhibition of NA synthesis affects SIA significantly.

In the present study we have demonstrated the antinociceptive activity of clonidine in the mouse using two different noxious stimuli and of phenylephrine in the abdominal constriction test. Selective antagonists have been used to investigate the involvement of α-adrenoceptors in three models of SIA in the mouse. Confirmation of a role for NA in SIA in the mouse was obtained by the measurement of brain NA levels of stressed mice in the naloxone sensitive model.

## Materials and Methods

### *Animals*

Male LACA mice (25–35 g) housed at 22 ± 2°C with lights on from 07.00 to 21.00 h, and with free access to food and water, were allowed to acclimatize for at least 90 min in the laboratory before experimentation between 11.00 and 17.00 h.

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*Nociceptive tests*

Two methods were used to assess the degree of antinociception produced by drugs or by stress. The abdominal constriction test involved the intraperitoneal (i.p.) injection of acetic acid (0·6% w/w, 10 mL $kg^{-1}$) with the abdominal constrictions counted between 10 and 20 min after the injection of the acetic acid. Potential antinociceptive agents were administered 15 min before acetic acid and antagonists 10 min before the antinociceptive agent, both by the i.p. route.

In the hot-plate test the mouse was placed on a surface maintined at 56°C for a maximum of 45 s or until the lifting or shaking of a hind paw was observed; movement of mice on the hot-plate was limited by a plexiglass cylinder (26 cm high and 20 cm diameter). An initial or screen reaction time ($T_1$) was obtained for each mouse and only those responding within 10 s were used. Potential antinociceptive agents were injected either by the i.p. or the intracerebroventricular (i.c.v.) route 30 min after the initial reaction time had been measured and the effect of the agent determined 15 or 10 min later. In mice treated with 6-hydroxydopamine (6-OHDA) (60 μg at 48 h intervals i.c.v), reaction times were determined 14 days after the screen reaction time.

*Stress-induced antinociception*

Three models of SIA were investigated which differed in the intensity of the stress, the method of assessment of antinociception and in their sensitivity to naloxone. Two of these have been described previously (Hart et al 1983) and are summarized as follows. (1) Mice were placed in a water bath (57 × 30 × 19 cm) at 30°C (i.e. warm water swim, WWS) for 30 s with antagonists or 0·9% NaCl (saline) injected (i.p.) 15 min before the stress. On removal from the water the mice were injected with acetic acid (as described above) and abdominal constrictions counted for 10 min after 10 min delay; (2) Mice were stressed in a water bath for 3 min at a water temperature of 20°C (i.e. room temperature swim, RTS). Hot-plate reaction times were determined three times: 30 min before the administration of saline, agonist or antagonist ($T_1$); 15 min later, which was immediately before the stress ($T_2$), and 2 min after the stress ($T_3$). The 2 min delay between the end of the stress and the measurement of a reaction time was occupied by the drying of the mouse, i.e. being towel dried, placed on absorbent paper in a mouse cage and warmed under a 60 W lamp. SIA was expressed as the difference between the post- ($T_3$) and pre-stress ($T_2$) values for reaction times. In the third model, mice swam for the same duration as in the second model but with water temperature reduced to 2°C (i.e. cold water swim (CWS)) thereby increasing the severity of the forced swim stress. Hot-plate tests were carried out pre- and post-CWS as in the second model, but were continued at 10 min intervals for up to 1 h after the first post-CWS measurement.

*Body temperature measurements*

In mice subjected to CWS, core body temperatures were measured with a thermistor probe (Light Laboratories) lubricated with petroleum jelly and inserted 2 cm into the rectum until a stable temperature (0·1°C accuracy) was obtained. Temperature measurements were made immediately after hot-plate reaction times were determined.

*Biochemical analysis*

Brain NA levels were determined in four groups; unstressed and stressed (3 min swim at 20°C or RTS) animals which had received 6-OHDA (60 + 60 μg i.c.v.) and equivalent controls which had received vehicle (1% ascorbic acid in saline). Immediately after the third ($T_3$) nociceptive threshold assessment on the hot-plate, each mouse was decapitated and the whole brain (minus cerebellum) was rapidly removed. Each brain sample was weighed and homogenized in ice-cold 0·1 M perchloric acid containing 0·23 M ascorbic acid and centrifuged at 14 500 rev $min^{-1}$ for 15 min, at 4°C. NA content was determined by HPLC using the method of Westerink (1984).

*Drugs*

Drugs used were clonidine HCl (Boehringer Ingelheim), idazoxan HCl (Reckitt & Colman), indoramin HCl (Wyeth), acetic acid, L-ascorbic acid and perchloric acid (BDH), 6-hydroxydopamine HCl, (−)-noradrenaline bitartrate, phenylephrine HCl and yohimbine HCl (Sigma), prazosin HCl (Pfizer) and naloxone HCl (Endo Lab.). All drugs were dissolved in saline before injection. Prazosin HCl was suspended in saline and subjected to ultrasonication in a water bath to improve the suspension. Indoramin HCl was dissolved in 1% ascorbic acid in saline as its vehicle. In some experiments using the hot-plate, drugs or vehicle were administered i.c.v. using a modification of the method described by Haley & McCormick (1957) with the injection volume not exceeding 5 μL. Coded solutions were used throughout the investigation with at least six mice per experimental group. The investigator was not allowed access to the code until after the completion of the experiment.

*Statistics*

Results from both the hot-plate test (change in reaction times) in RTS experiments and from the abdominal constriction test (number of constrictions in 10 min period) are expressed as medians with interquartile ranges ($Q_1$–$Q_3$) for each group. Comparisons between groups was assessed by the appropriate test; either the Kruskal-Wallis test or the Mann-Whitney test. Results from measurement of brain NA levels and temperature measurements of animals subjected to CWS were analysed by Student's *t*-test, and temperature measurements were also subjected to analysis of variance (ANOVA). A probability of $\leq 0{\cdot}05$ was assumed to denote a significant difference between control and test groups.

## Results

*Effects of clonidine, phenylephrine and α-adrenoceptor antagonists on acetic acid-induced abdominal constriction and WWS SIA*

In saline-pretreated mice the injection of acetic acid caused a median of 26·5 abdominal constrictions. Clonidine, at doses of 12·5, 25 and 50 μg $kg^{-1}$, produced a dose-related reduction in the number of constrictions (Table 1) and this action of clonidine was reduced by yohimbine (1 mg $kg^{-1}$ Fig. 1). Phenylephrine (5–10 mg $kg^{-1}$) also produced dose-dependent inhibition of the constrictions which was antagonized by indoramin (2 mg $kg^{-1}$). In mice exposed to the mild stress of a 30 s swim at 30°C, the number of constrictions induced by acetic acid was reduced from 26·5 to 6 ($P = 0{\cdot}001$). This

Table 1. Effects of clonidine, phenylephrine (PE), indoramin, prazosin, idazoxan and yohimbine on the acetic acid-induced abdominal constrictions and WWS SIA. Each dose was administered via the i.p. route 15 min before acetic acid injection. Indoramin was given 10 min before phenylephrine. Clonidine, phenylephrine and prazosin dose-dependently and significantly ($P<0.01$) reduced the number of abdominal constrictions while idazoxan and yohimbine were without effect on the unstressed animals but significantly ($P<0.01$) reversed the WWS SIA in stressed mice. Indoramin was without effect alone but reversed phenylephrine induced inhibition of the constrictions. Median constrictions with interquartile ranges ($Q_1$–$Q_3$) are shown in parentheses per dose group (n = 6). Data were analysed with the Kruskal-Wallis test against appropriate control. # is $P=0.001$ against unstressed saline control using the Mann–Whitney test.

| Drug and dose | Unstressed mice Constrict. Median ($Q_1$–$Q_3$) | *P* vs cont. | Stressed mice Constrict. Median ($Q_1$–$Q_3$) | *P* vs cont. |
|---|---|---|---|---|
| Saline (10 mL kg$^{-1}$) | 26·5 (26–29) | | | |
| Clonidine (12·5 μg kg$^{-1}$) | 19 (17–20) | <0·01 | | |
| (25 μg kg$^{-1}$) | 9 (5–12) | <0·01 | | |
| (50 μg kg$^{-1}$) | 6 (3–9) | <0·01 | | |
| PE (5 mg kg$^{-1}$) | 14 (9–16) | <0·01 | | |
| (10 mg kg$^{-1}$) | 10 (6–13) | <0·01 | | |
| Vehicle (10 mL kg$^{-1}$) | 26 (25–29) | | 6·5 (6–9) | |
| Indoramin (1 mg kg$^{-1}$) | 27 (24–29) | | 7 (5–9) | |
| (2 mg kg$^{-1}$) | 27·5 (26–30) | | 6·5 (4–8) | |
| Vehicle (10 mL kg$^{-1}$) + PE (10 mg kg$^{-1}$) | 12 (7–15)# | | | |
| Indoramin (2 mg kg$^{-1}$) + PE (10 mg kg$^{-1}$) | 26 (20–28)# | 0·001 | | |
| Saline (10 mg kg$^{-1}$) | 27 (25–29) | | 6 (5–10)# | |
| Prazosin (1 mg kg$^{-1}$) | 14 (9–16) | <0·01 | | |
| (2 mg kg$^{-1}$) | 5 (0–8) | <0·01 | | |
| Idazoxan (0·5 mg kg$^{-1}$) | 27 (24–29) | | 22·5 (20–24) | <0·01 |
| (1 mg kg$^{-1}$) | 27 (23–30) | | 26 (23–26) | <0·01 |
| Yohimbine (0·5 mg kg$^{-1}$) | 28 (25–29) | | 25 (23–27) | <0·01 |
| (1 mg kg$^{-1}$) | 28 (27–30) | | 26 (24–28) | <0·01 |

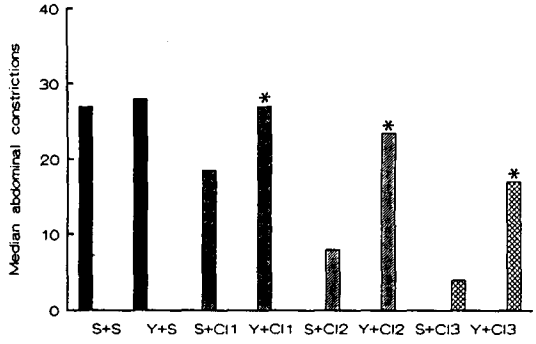


FIG. 1. Reduction of clonidine-induced antinociception by yohimbine. Mice pretreated with saline produced a median of 27 abdominal constrictions which was not significantly affected by yohimbine (1 mg kg$^{-1}$). Yohimbine however, significantly ($P<0.001$) antagonized clonidine (12·5–50 μg kg$^{-1}$) induced inhibition of the writhes. Mice were given saline or yohimbine 10 min before clonidine, which was administered 15 min before acetic acid injection. All agents were given via the i.p. route. Data were analysed by the Mann–Whitney test (n = 6 per bar). S = saline (10 mL kg$^{-1}$), Y = yohimbine (1 mg kg$^{-1}$), C11 = clonidine (12·5 μg kg$^{-1}$), C12 = clonidine (25μg kg$^{-1}$) and C13 = clonidine (50 μg kg$^{-1}$).

SIA was attenuated in mice pretreated with either yohimbine or idazoxan (Table 1). Both these $\alpha_2$-antogonists produced some abdominal constrictions on injection but did not affect the number of constrictions produced by acetic acid in non-stressed control animals. In contrast, prazosin (1, 2 mg kg$^{-1}$) reduced the number of constrictions due to acetic acid and appeared to depress the locomotor activity of the mice. For these reasons mice treated with prazosin were not stressed. Indoramin produced no such effects and stressed mice treated with this drug showed antinociception similar to that of saline-treated animals (Table 1).

*Effects of i.c.v. injection of NA and i.p. injections of clonidine and α-adrenoceptor antagonists on hot-plate latencies and RTS SIA*

Exposure of mice to a 3 min swim at 20°C (RTS) increased the hot-plate latency from 0·5 to 12 s. NA alone (5 and 10 μg i.c.v.) did not influence reaction times but in mice pretreated with the higher dose of NA, and stressed, the duration of the subsequent antinociception increased to 18 s (Table 2). Clonidine at 0·5 mg kg$^{-1}$ produced an even more dramatic increase in SIA but at a dose which alone had a small, but significant ($P<0.01$), antinociceptive action. The ability of clonidine to augment SIA was sensitive to yohimbine (1 mg kg$^{-1}$). Prazosin, idazoxan and yohimbine had no effect on hot-plate latencies in unstressed mice but each antagonist reduced RTS SIA significantly (Table 2).

*Effects of 6-OHDA on hot-plate latency, RTS SIA and on NA levels*

The effectiveness of the α-adrenoceptor antagonists in reducing RTS SIA suggests a role for NA as a mediator in this model of SIA. To examine this hypothesis a direct investigation was made in mice in which endogenous stores of brain NA were depleted by 6-OHDA. Mice appeared to be hypersensitive on the hot-plate (Table 3) after treatment with

Table 2. Effects of NA, clonidine, prazosin, idazoxan and yohimbine on hot-plate latency and on RTS SIA. The saline vehicle and NA were given by the i.c.v. route 10 min before swim or control procedure. Clonidine, prazosin, idazoxan and yohimbine were administered intraperitoneally 15 min before swim or control procedure. In the antagonism experiments, saline or yohimbine was injected 10 min before clonidine (0·5 mg $kg^{-1}$). Median differences in the reaction times between post no-swim ($T_3$) and screen ($T_1$) values for unstressed mice, as well as between post-swim ($T_3$) and pre-swim ($T_2$) values for stressed mice are shown with interquartile ranges ($Q_1$–$Q_3$) in parentheses per dose group (n = 6). Data were analysed with the Kruskal–Wallis test against appropriate control. # is $P = 0.001$ using the Mann–Whitney test against appropriate control.

| | Unstressed mice ($T_3$–$T_1$) s | | | Stressed mice ($T_3$–$T_1$) s | | |
|---|---|---|---|---|---|---|
| Drug and dose | Median | ($Q_1$–$Q_3$) | *P* vs cont. | Median | ($Q_1$–$Q_3$) | *P* vs cont. |
| Saline (5 μL i.c.v.) | 0·5 | (0–1) | | 12 | (10–13) | |
| NA (5 μg i.c.v.) | 0·25 | (0–1) | | 12·5 | (11–13·5) | |
| (10 μg i.c.v.) | 0 | (0–1) | | 18 | (13–20) | < 0·05 |
| Saline (10 mL $kg^{-1}$) | 1 | (0–1·5) | | 13 | (11–16)# | |
| Clonidine (0·5 mg $kg^{-1}$) | 2·5 | (2–4) | < 0·01 | 39 | (34–41) | < 0·01 |
| Prazosin (1 mg $kg^{-1}$) | 1 | (0–1) | | 7 | (4–9) | < 0·01 |
| (2 mg $kg^{-1}$) | 0·5 | (0·5–2) | | 4 | (4–6) | < 0·01 |
| Idazoxan (0·5 mg $kg^{-1}$) | 0 | (0–1) | | 5·5 | (4·5–8) | < 0·01 |
| (1 mg $kg^{-1}$) | 0·5 | (0·5–1) | | 6 | (4–6) | < 0·01 |
| Yohimbine (0·5 mg $kg^{-1}$) | 0·5 | (0–1) | | 7 | (5–8·5) | < 0·01 |
| (1 mg $kg^{-1}$) | 0 | (0–1·5) | | 3 | (2–5) | < 0·01 |
| Saline (10 mg $kg^{-1}$ +) | | | | | | |
| Clonidine (0·5 mg $kg^{-1}$) | | | | 34 | (30–36) | |
| Yohimbine (1 mg $kg^{-1}$ +) | | | | | | |
| Clonidine (0·5 mg $kg^{-1}$) | | | | 13·5 | (11–16·5)# | < 0·001 |

Table 3. Effect of i.c.v. administration of 6-OHDA on hot-plate latency and RTS SIA. Reaction times ($T_3$ and $T_2$) of mice in unstressed and stressed conditions were determined 14 days after the screen ($T_1$) reaction time. 6-OHDA was administered twice; first 60 μg on day 1 and 48 h after, another 60 μg was injected. Median differences in the reaction times between post no-swim ($T_3$) and screen ($T_1$) values for unstressed mice as well as between post-swim ($T_3$) and pre-swim ($T_2$) values for stressed mice are shown with interquartile ranges ($Q_1$–$Q_3$) in parentheses where n = 8 per group (# = n = 6). Data were analysed with the Mann–Whitney test.

| | Unstressed mice ($T_3$–$T_1$) s | | | Stressed mice ($T_3$–$T_1$) s | | |
|---|---|---|---|---|---|---|
| Drug and dose | Median | (Q1–Q3) | *P* vs cont. | Median | ($Q_1$–$Q_3$) | *P* vs cont. |
| Vehicle 5 μL + 5 μL | 0 | (−1–0·5) | | 11·5 | (10·25–12·5) | |
| 6-OHDA 60 μg + 60 μg | −3 | (−3·5– −3)# | < 0·001 | 5·5 | (4–7·75) | < 0·001 |

6-OHDA. The nociceptive thresholds of both unstressed and stressed animals treated with 6-OHDA were significantly ($P < 0.001$) lowered when compared with values from the controls. Examination of NA levels in Fig. 2 reveals a significant ($P < 0.001$) reduction (17·5%) in brain NA levels of stressed mice when compared with unstressed animals pretreated with the vehicle. The mean NA concentration in unstressed animals pretreated with 6-OHDA was significantly less (39%; $P < 0.001$) than that of mice pretreated with its vehicle but was significantly ($P = 0.05$) higher than that of stressed animals which received this neurotoxin.

*Effects of i.c.v. injections of NA, naloxone, prazosin and yohimbine on CWS SIA and hypothermia*

The stress of a 3 min swim in cold water (CWS) initially increased hot-plate latencies to beyond the 45 s cut-off time and the SIA continued for 40 min (Fig. 3a). Yohimbine (5 μg i.c.v.) had no effect in unstressed mice but reduced SIA at all time intervals. In contrast, naloxone (5 μg i.c.v.) and

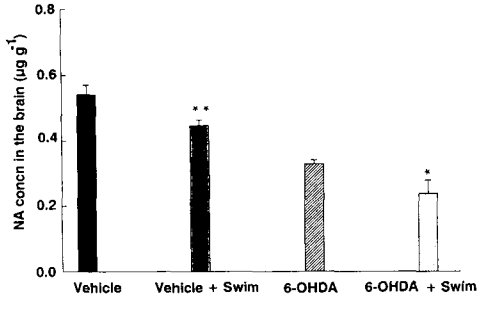


FIG. 2. Effect of room temperature swim (RTS) on brain NA levels and the influence of 6-OHDA. Mean (± s.e.m.) concentrations (μg $g^{-1}$ tissue) of NA in the brain (minus cerebellum) of mice killed immediately after non-stressed (control) and RTS SIA determination at 14 days after the injection of the vehicle (1% ascorbic acid in saline of 2 × 5 μL i.c.v.) and 6-OHDA (2 × 60 μg i.c.v.). RTS at 20 °C significantly ($P < 0.001$) reduced brain NA levels in vehicle and 6-OHDA treated animals. 6-OHDA itself significantly ($P < 0.001$) reduced the NA levels when compared with its vehicle without the swim. Data were analysed with the Student's *t*-test and n = 8 per bar except for that of 6-OHDA where n = 6. * $P = 0.05$; ** $P < 0.001$.

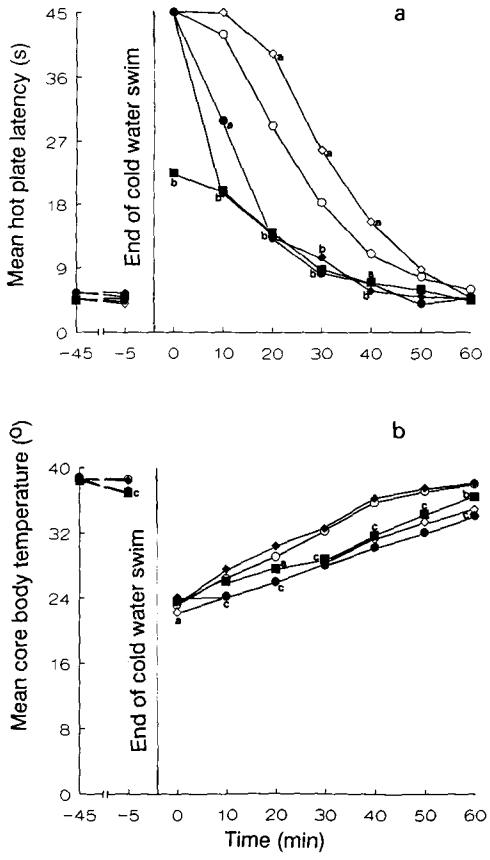


FIG. 3. Effects of NA, naloxone, prazosin and yohimbine on (a) CWS SIA, and (b) CWS induced hypothermia. (a) Mice given saline (5 μL) reached the cut-off time of 45 s at 0 min (2 min post-CWS) and produced a time dependent decrease in SIA at subsequent 10 min intervals. NA (10 μg i.c.v.) treated mice also reached the cut-off point at the first and second post-CWS SIA determinations, and enhanced CWS SIA significantly ($P < 0.05$) at further intervals until 40 min (42 min after CWS). Naloxone (5 μg i.c.v.) and prazosin (5 μg i.c.v.) were also without effect on the cut-off threshold 2 min after the swim, but reduced CWS SIA at other test times. Only yohimbine (5 μg i.c.v.) significantly ($P < 0.01$) lowered the nociceptive threshold at the first post-CWS SIA measurement and at subsequent intervals. All drugs were without effect on pre-CWS hot-plate latencies. Each datum point represents the median value (n = 6). Drug treated groups were statistically compared against saline controls using the Kruskal-Wallis test. (b) All drugs were given via the i.c.v. route approximately 10 min before CWS. Naloxone (5 μg) is shown to be without significant effect pre-CWS and post-CWS. Prazosin (5 μg), yohimbine (5 μg) and NA (10 μg) produced hypothermia ($P < 0.001$) when core body temperatures were measured 10 min post injection or pre-CWS. NA, prazosin and yohimbine potentiated CWS-induced hypothermia from the 0 min (2 min post-CWS), 10 min (12 min post-CWS) and 20 min (22 min post-CWS), respectively, until the end of the test period. Each data point represents the mean temperature measurement where n = 6 per drug group (as in a). Drug treated groups were statistically compared with saline controls using Student's *t*-test subsequent to ANOVA. Key ○ saline, 5 μL; ◇ NA, 10 μg; ● prazosin, 5 μg; ■ yohimbine, 5 μg; ◆ naloxone, 5 μg; a $P < 0.05$; b $P < 0.01$; c $P < 0.001$.

prazosin (5 μg i.c.v.) inhibited only the later stages of this SIA. A dose of NA (10 μg i.c.v.), which had no effect on reaction times of unstressed mice, produced a significant increase in the hot-plate latencies at 20–40 min ($P < 0.05$) after the first measurement (Fig. 3a).

Core body temperatures of stressed mice returned to normal after 60 min. The changes in temperature produced by the antagonists did not parallel their effects on CWS SIA. Naloxone, although reducing SIA, had no effect on body temperature whilst prazosin and yohimbine produced significant ($P < 0.001$) hypothermia alone and increased that occurring in stressed mice at a time when they inhibited SIA. NA also caused hypothermia and enhanced that produced by the CWS at a time when it was also enhancing CWS SIA (Fig. 3b).

## Discussion

The present study confirms that clonidine and PE are antinociceptive in the mouse abdominal constriction test and that clonidine is also active in the hot-plate test. The use of selective antagonists indicates that $\alpha_2$-adrenoceptors are involved in the three models of swim SIA. Idazoxan and yohimbine induced writhing activity in mice, in the absence of the irritant, but did not influence the number of abdominal constrictions induced by acetic acid injection. The selective $\alpha_1$-antagonist, prazosin, produced antinociception which is unlikely to be mediated through adrenoceptors but may be due to some other cause, such as local anaesthetic action, since the animals treated with this drug appeared sedated. A similar suggestion was made by Bentley et al (1983) who found that prazosin did not antagonize NA-induced antinociception. Indoramin, which has $\alpha_1$-antagonist activity similar to prazosin (Ramage 1986), had no significant effect on both unstressed and WWS-stressed animals at a dose which antagonized PE-induced antinociception. This implies that $\alpha_1$-adrenoceptors are not involved in this stress model. That idazoxan and yohimbine reversed the WWS induced reduction in the number of the abdominal constrictions suggests that $\alpha_2$-adrenoceptors are involved in this model of SIA which has been shown by Hart et al (1983) to be naloxone-insensitive and, therefore, a non-opioid model of SIA.

Although recent data have shown that clonidine has effects at both $\alpha_1$- and $\alpha_2$-adrenoceptors, Hayes et al (1986b) concluded that antinociception induced by clonidine is mediated predominantly by $\alpha_2$-adrenoceptors which is confirmed in the present study where clonidine-induced increase in the hot-plate latency is antagonized by yohimbine. Early studies by Schmitt et al (1974) showed that xylazine-induced increases in the reaction latency on the hot-plate were attenuated by yohimbine but not by phenoxybenzamine, consistent with an effect at $\alpha_2$-adrenoceptors. That 6-OHDA produced shorter reaction times, or hyperalgesia, on the hot-plate in the present study is in accord with the results of other workers (Slater 1974; Slater & Blundell 1978). Its reduction of RTS SIA could be due to central nervous system (CNS) NA depletion by this neurotoxin, i.e. the destruction of a significant proportion of the neurons which subserve antinociception and SIA.

The involvment of NA in the neurochemical consequences of stress is verified by lower levels of this catecholamine (in the brain) measured after the RTS. This confirms the work of Stone (1973). Zacharko & Anisman (1984) have proposed that when animals are exposed to a stressor, they seek behavioural means of coping and that this may be facilitated by increased central amine synthesis and utilization. Equally in support of their proposal, when behavioural methods of coping are unavailable, or perceived as being unavailable, during inescapable stress such as the 3 min swim, greater

demands will be placed on endogenous neurochemical systems in which the utilization of NA is more likely to exceed its synthesis so that its concentration falls. This depletion is said to vary not only with the stress severity, but also with the brain region (Nakagawa et al 1981). The antinociceptive response to CWS also appears to correlate with changes in brain NA levels since acute exposure of rats to CWS reduced brain NA (Stone 1970) and induced analgesia (Bodnar et al 1978). Foot-shock stress has also been shown to reduce brain NA (Thierry et al 1968) and to elicit analgesia sensitive to yohimbine (Chance & Schechter 1979).

The enhancing effect of NA on RTS SIA appears to be mediated by both $\alpha_1$- and $\alpha_2$-adrenoceptors since prazosin, idazoxan and yohimbine reduced the resulting antinociception. In addition, clonidine-induced potentiation of RTS SIA was abolished by yohimbine. Belcher et al (1978) showed that NA has a post-synaptic inhibitory action on many dorsal horn neurons including the thermal nociceptive neurons. Their result may explain why prazosin also inhibited naloxone-sensitive RTS SIA (Hart et al 1983) and confirms the preliminary report of Hart & Yadav (1985). That NA potentiated antinociception resulting from RTS and CWS, and enhanced CWS-induced hypothermia, appears to be consistent with the result of Bodnar et al (1983) who showed clonidine's augmentation of CWS-induced analgesia and hypothermia. It appears that NA mediates its effect predominantly through $\alpha_2$-adrenoceptors by providing excitation of the nociceptive inhibitory system and that $\alpha_1$-adrenoceptors are also involved as described above. Further evidence is suggested by yohimbine-induced inhibition of the apparent naloxone-insensitive or non-opioid component of CWS SIA determined 2 min post-CWS (Fig. 3a). Naloxone significantly reduced the thermal latency at subsequent intervals of nociceptive response determinations. This appears to be a period during which the significance of the non-opioid stress activated system decreases and the role of opioid systems emerge. Yohimbine's attenuation of CWS SIA is however in contrast to the results of Kepler & Bodnar (1988) who reported yohimbine-potentiation of CWS analgesia in the rat after i.p. administration. The different routes of yohimbine administration, as well as the nociceptive test and species difference, may account for the opposing results. The reduction of both RTS and CWS SIA by yohimbine suggests that it may be removing descending inhibitory influences to the spinal cord. Since $\alpha_2$-adrenoceptors are inhibitory to the release of NA, the attenuatory action of idazoxan and yohimbine on SIA is possibly linked to increased release and neuronal activity of NA in the CNS. In support of this interpretation, Westerink (1984) reported that 5 mg $kg^{-1}$ of yohimbine increased NA turnover in several brain areas; but after the injection of clonidine, there were increased levels of NA in brain samples. In the present study the inhibitory effect of yohimbine on the apparent naloxone-insensitive component of CWS SIA, seems to support the work of Aimone et al (1987). They demonstrated that the adrenoceptor involved in stimulation-produced analgesia from the brain NRM was $\alpha_2$ due to its inhibition by intrathecal administration of yohimbine but not by prazosin or naloxone. Other studies have shown that yohimbine reduces opioid-mediated prolonged intermittent foot-shock analgesia, non-opioid-mediated brief continous foot-shock analgesia and autoanalgesia (Chance & Schechter 1979; Coderre & Rollman 1984; Chance 1986).

The NA induced hypothermic response in the mouse confirms the report of Brittain & Handley (1967). The hypothermic effect of yohimbine shown in this study has been reported earlier by Sanghvi & Gershon (1974). The mechanism of this hypothermia is not clear; though it is believed to be mediated peripherally. If the manipulation that alters CWS SIA is acting on the stressful consequences of CWS itself, it would be expected that parallel changes in the antinociceptive and hypothermic responses to CWS would occur. In this regard, both CWS-induced analgesia and hypothermia were reduced in rats neonatally treated with monosodium glutamate (Onley 1969; Badillo-Martinez et al 1982) which is a non-invasive means of producing relative destruction of the medial basal hypothalamus through its neurotoxic effects. Bodnar (1986) has suggested that monosodium glutamate treatment produced more general changes in an animal's physiological responses to stress, rather than a selective effect upon a given endogenous pain inhibitory system. The potentiating action of NA on both antinociceptive and hypothermic responses following CWS in the present study shows that intracerebral NA enhanced the stressful consequences of the CWS. A similar observation has been made with clonidine (Bodnar et al 1983). In the present study however, yohimbine also potentiated the hypothermic effect of the CWS but inhibited its antinociceptive effect. In conclusion, noradrenergic pathways are involved in the antinociception resulting from each of the swim stresses. $\alpha_2$-Adrenoceptors are involved in each model whilst the non-involvement of $\alpha_1$-adrenoceptors in the WWS may be associated with the method used to assess antinociception.

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