Mechanisms Mediating the Thermal Response to Morphine Withdrawal in Rats

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MICHAEL J. KATOVICH,* DAVID L. PITMAN,* AND CHRISTOPHER C. BARNEY†

Department of Pharmacodynamics, College of Pharmacy University of Florida, Gainesville, Florida 32610 and Department of Biology,† Hope College, Holland, Michigan 49432*

Abstract. **Studies were undertaken to evaluate the role of peripheral adrenergic mechanisms and the adrenal gland in the thermal responses which accompany morphine withdrawal in the rat. Ovariectomized rats were addicted to morphine and subsequently withdrawn by administration of naloxone. This treatment resulted in a significant rise (5–6°C) in tail skin temperature (TST) and fall in colonic temperature (2–4°C). Systemic administration of clonidine (0.5 mg/kg) completely suppressed this surge in TST and significantly attenuated the fall in core temperature. Similar results were observed following the systemic administration of ST-91, another α_2-adrenergic agonist which does not cross the blood-brain barrier. Central administration of ST-91 (50 μg/5 μl, icv) was also successful in attenuating these temperature changes in the morphine-dependent rat. Adrenalectomy and peripheral administration of propranolol (10 mg/kg sc) both resulted in a significant attenuation of the surge in TST and the fall in core temperature in the morphine-dependent rat which suggest some peripherally mediated event is necessary to produce the full skin temperature surge. Collectively, the data suggest a role for the adrenal gland and adrenergic receptors in producing the surge in TST in morphine-dependent rats. It also suggests that the blocking effects of the α_2-adrenergic agonist can be mediated both centrally and peripherally.** [P.S.E.B.M. 1990, Vol 193]

The interaction between morphine and catecholamine metabolism was first suggested by Gunne (1) when he reported that chronic administration of morphine affected norepinephrine content in rat brains. Swann *et al.* (2) later demonstrated that naloxone-precipitated withdrawal in rats resulted in an increase in brain norepinephrine turnover. It has been suggested that the noradrenergic locus coerulous neurons become hyperactive during naloxone-precipitated morphine withdrawal, resulting in an increased firing rate (3). Collectively, these studies suggest that central catecholamine release may be important in initiating and maintaining opiate withdrawal. Adrenal catecholamine activity has also been reported to be altered by opiates (4, 5) and morphine withdrawal results in a selective release of epinephrine from the adrenal medulla (4). Distefano and Brown (5) suggested that this selective release of epinephrine has both a direct and a centrally mediated component.

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This correlation between increased noradrenergic activity and opiate withdrawal is also consistent with the observations that clonidine, an α_2-adrenergic agonist, reduces the severity of the signs and symptoms of opioid withdrawal in humans (6) and in morphine-dependent rats (7). The anti-withdrawal effects of clonidine have been suggested to be a result of suppression of the increased adrenergic activity and presynaptic norepinephrine release (3, 8). Biochemical studies have also demonstrated that the increase in catecholamine turnover observed during morphine withdrawal can be prevented by clonidine (9, 10). In addition, Distefano and Brown (10) demonstrated that clonidine blocked the depletion of epinephrine from the adrenal gland and reduced the increased adrenal tyrosine hydroxylase activity associated with morphine withdrawal.

We have recently implicated a role for central noradrenergic neurons in the tail skin temperature (TST) surge that accompanies morphine withdrawal in the rat (11). Brent *et al.* (4) recently reported an elevation in plasma epinephrine following naloxone precipitated withdrawal in morphine-treated guinea pigs which is absent in adrenalectomized animals. In the present study we therefore determined if there were any peripheral components involved in the TST surge as-

sociated with morphine withdrawal. Studies were conducted to evaluate the effects of adrenalectomy, clonidine, and ST-91, another α_2-agonist which cannot cross the blood-brain barrier (12, 13), on the TST surge in order to evaluate central versus peripheral control of this response. Since propranolol treatment has been shown to reduce the elevated TST associated with isoproterenol administration in rats (14), we also evaluated if there is a β-adrenergic mediated component of the TST response to morphine withdrawal.

Materials and Methods

Animals and Morphine Treatment. For all studies female Charles Rivers CD rats initially weighing 225–250 g were housed in pairs in hanging stainless steel cages in a room maintained at 25 ± 1°C and illuminated from 0500 to 1900 hr. Food and tap water were provided *ad libitum*. Since we have previously reported that estrogens can modify the temperature response (15), rats were ovariectomized while under light ether anesthesia 1 week prior to morphine dependency.

Morphine dependency was produced in the rats by subcutaneous implantation of one pellet containing 75 mg of morphine-free base (Merck, St. Louis, MO), 1.13 mg of magnesium sterate (Fisher Chemical Co., Fair Lawn, NJ), 37.5 mg of microcrystalline cellulose (Avicel; FMC Corporation, Philadelphia, PA), and 0.56 mg of Cab-o-sil (Cabot Corp., Boston, MA). Two days later, two additional morphine pellets were implanted. All pellets were compounded in our laboratory using a F. J. Stokes tablet maker. This treatment regimen has been shown previously to produce morphine dependency as measured by several testing procedures (7, 16). In all studies, animals were used once only and then sacrificed by decapitation.

Temperature Studies. Two days after the second implantation of morphine, the animals were lightly restrained in wire mesh tunnel cages with a wooden floor in a room kept at 26 ± 1°C. TST was measured with a copper-constantan thermocouple that was taped to the dorsal region of the tail. Colonic temperature (Tc) was measured with a copper-constantan thermocouple inserted 6 cm beyond the anus and taped to the base of the tail. Temperatures were recorded at 5-min intervals with a data acquisition and control system (Cyborg) interfaced to an Apple IIe computer. The drug of interest or a control solution was administered 1 hr after the rats were placed in the restraining cages. Thirty minutes later all animals were administered naloxone HCl (1 mg/kg sc, Sigma) and tail skin and colonic temperatures were recorded for an additional 60 min. Previous studies have shown that neither subcutaneous saline administration to morphine-dependent rats nor naloxone administration to placebo-treated rats produce any changes in tail skin or colonic temperature (16, 17).

For all experiments each group consisted of six rats. The control rats for each experiment received 0.9% NaCl solution by the same route (1 ml/kg sc or 5 μl icv) as the test drug. In Experiment 1, the experimental group of rats received clonidine HCl (0.5 mg/kg sc; Sigma). In Experiment 2, the experimental group received ST-91 (Boehringer Ingelheim Laboratories) at doses of 0.5 mg/kg or 3.0 mg/kg sc. ST-91 (2-(2,6-diethylphenylimino)-2-imidazolidine) is a peripheral acting α-adrenergic agonist which does not cross the blood-brain barrier (12, 13). In Experiment 3, the experimental group of rats received 50 μg of ST-91 administered into a lateral cerebral ventricle in 5 μl of saline.

For the intracerebroventricular (icv) injections rats were anesthetized with sodium pentobarbital (30 mg/kg ip), placed in a Kopf stereotaxic apparatus, and implanted with a single cannula (26-gauge stainless steel tubing; Small Parts, Miami, FL) in the lateral ventricle 4 days before the initiation of morphine treatment. The coordinates, obtained from the stereotaxic atlas of König and Klippel (18), were anterior +5.0 mm, lateral 0.5 mm, and ventral 7 mm below the surface of the skull. A wire stylet was fitted into the outer guide cannula and remained in place until the temperature experiments were performed as described previously (11). Following the experiments rats were sacrificed and brains were dissected to verify the location of the cannula.

In Experiment 4, propranolol HCl (Sigma) was administered to the experimental group (10 mg/kg sc). In Experiment 5, on the same day that all rats received the first morphine pellet, one half of the rats were adrenalectomized and one half underwent sham adrenalectomy while under light ether anesthesia. Two days later all rats were implanted with two additional morphine pellets. All animals in Experiment 5 were maintained on a 1% NaCl drinking solution. Four days after the first morphine implant, TST and colonic temperature responses to naloxone without a prior drug treatment were monitored.

For all studies involving two groups of rats, basal and mean changes in TST and Tc were compared using Student's t tests for each time interval before and after drug administration. If more than two groups were utilized, a one-way analysis of variance followed by a Newman-Keuls test was used to evaluate the significance of differences among treatment groups. Data were also summarized as area under the TST curve. Significance was set at the 95% probability level. All data are expressed as mean ± SE.

Results

Basal Tc and TST were similar in both the experimental and control groups before and after administration of the test drug in all experiments except that clonidine significantly lowered Tc (Table I). In Experiment 1, naloxone increased TST in the control group but not in the clonidine-treated group (Fig. 1). The integrated areas under the TST curves and maximal

Table I. Effect of Clonidine and ST-91 on the Rise in TST and the Fall in Tc associated with Naloxone Administration (1 mg/kg sc) to Morphine-Dependent Female Rats

Experimental groups	Basal[a] TST (°C)	Maximal change in TST (°C)	Area under 60 min TST curve (°C-min)	Basal[a] Tc (°C)	Maximal change in Tc (°C)	Area under 60 min Tc curve (°C-min)
Study 1						
Control	25.2 ± 0.4[b]	6.1 ± 0.3	275.5 ± 13.7	40.1 ± 0.3	−3.5 ± 0.4	−113.6 ± 12.6
Clonidine (0.5 mg/kg)	25.9 ± 0.5	−1.6 ± 0.4[c]	−57.3 ± 14.8[c]	38.2 ± 0.2[c]	−1.6 ± 0.2[c]	−40.6 ± 3.5[c]
Study 2						
Control	25.7 ± 0.5	4.6 ± 0.3	190.9 ± 21.2	39.7 ± 0.3	−4.7 ± 0.4	−143.2 ± 20.7
ST-91 (0.5 mg/kg)	24.3 ± 0.2	0.4 ± 0.8[c]	−7.4 ± 46.1[c]	38.7 ± 0.2	1.9 ± 0.4[c]	−41.9 ± 16.4[c]
ST-91 (3.0 mg/kg)	25.4 ± 0.3	−0.9 ± 0.3[c]	−16.9 ± 12.6[c]	39.3 ± 0.2	−2.8 ± 0.2[c]	−88.2 ± 8.1[c]
Study 3						
Control	24.8 ± 0.2	3.3 ± 0.9	143.5 ± 38.4	38.6 ± 0.3	−3.5 ± 0.3	−112.8 ± 14.3
ST-91 (50 μg/5 μl icv)	24.6 ± 0.3	−1.4 ± 0.5[c]	−51.8 ± 17.5[c]	38.0 ± 0.3	−1.9 ± 0.4[c]	−53.3 ± 12.4[c]
Study 4						
Control	26.3 ± 0.2	5.2 ± 0.3	228.3 ± 16.6	38.5 ± 0.2	2.8 ± 0.2	−100.9 ± 9.9
Propranolol (10 mg/kg)	26.7 ± 0.2	1.8 ± 0.5	76.9 ± 23.3[c]	37.9 ± 0.2	3.0 ± 0.2	−88.5 ± 6.4

[a] Refers to temperatures prior to administration of naloxone.
[b] Data expressed as mean ± 1 SE.
[c] Significantly different from respective control group (<0.05).

changes in TST are summarized in Table I and also demonstrate that systemic administration of clonidine attenuates the TST response to naloxone. Animals pretreated with clonidine displayed an attenuated decrease in Tc following administration of naloxone (Fig. 1 and Table I) as depicted in both maximal changes in temperature and area under the temperature curve. However, the actual colonic temperatures of the two groups (36.5 ± 0.2°C and 36.6 ± 0.3°C) were not significantly different at 60 min after administration of naloxone. All rats appeared to display similar withdrawal-like behaviors, although no quantitative measurements were undertaken.

In Experiment 2, systemic utilization of ST-91 produced a similar attenuation of the TST response to naloxone in morphine-dependent rats. Only the control group displayed an elevated TST response following administration of naloxone (Fig. 2 and Table I). Both doses of ST-91 significantly diminished the fall in Tc following administration of naloxone (Table I). When ST-91 was administered via a central route (50 μg/5 μl icv) in Experiment 3, it produced a similar attenuation of both the rise in TST and the fall in Tc associated with administration of naloxone in the morphine-dependent rat. This is shown in both the maximal temperature changes and area under their respective curves (Table I).

Figure 3 summarizes the temperature responses to naloxone when animals were pretreated with the β-adrenergic antagonist, propranolol. The surge in TST associated with naloxone treatment was significantly ($P < 0.05$) reduced in the propranolol-treated group as shown by both the maximal change and the area under the TST curve (Table I). Following administration of naloxone, both groups demonstrated a similar reduction in colonic temperature (Table I).

Figure 4 summarizes the difference in TST response to administration of naloxone in intact and 4-day adrenalectomized, morphine-dependent rats. Adrenalectomy did not alter basal TST between the control group (27.3 ± 0.2°C) and the experimental group (27.0 ± 0.3°C). However, the 2.3 ± 0.7°C rise in TST observed 15 min after administration of naloxone in the adrenalectomized rat was significantly lower than the 4.8 ± 0.5°C rise observed in the intact controls. The area under the TST curve also demonstrated a significant difference ($P < 0.05$) between control (223.2 ± 14.1°C-min) and adrenalectomized (95.6 ± 25.0°C-min) groups. Adrenalectomy resulted in a lower ($P < 0.05$) basal Tc of 37.6 ± 0.7°C when compared with the 39.9 ± 0.2°C observed in the control rats. Following administration of naloxone, the fall in Tc in the control group was significantly ($P < 0.05$) greater (−4.1 ± 0.2°C) than the fall observed in the adrenalectomized group (−2.6 ± 0.4°C). However, the absolute Tc at the conclusion of the study were similar in the control (35.7 ± 0.2°C) and adrenalectomized (34.9 ± 0.4°C) groups. The area under the Tc curve of the control group (−133.6 ± 6.2 °C-min) was significantly ($P < 0.05$) greater than that observed in the adrenalectomized group (−83.6 ± 8.4°C-min).

Discussion

We have demonstrated previously that administration of naloxone to morphine-dependent rats induces a TST surge in concert with other physiologic responses

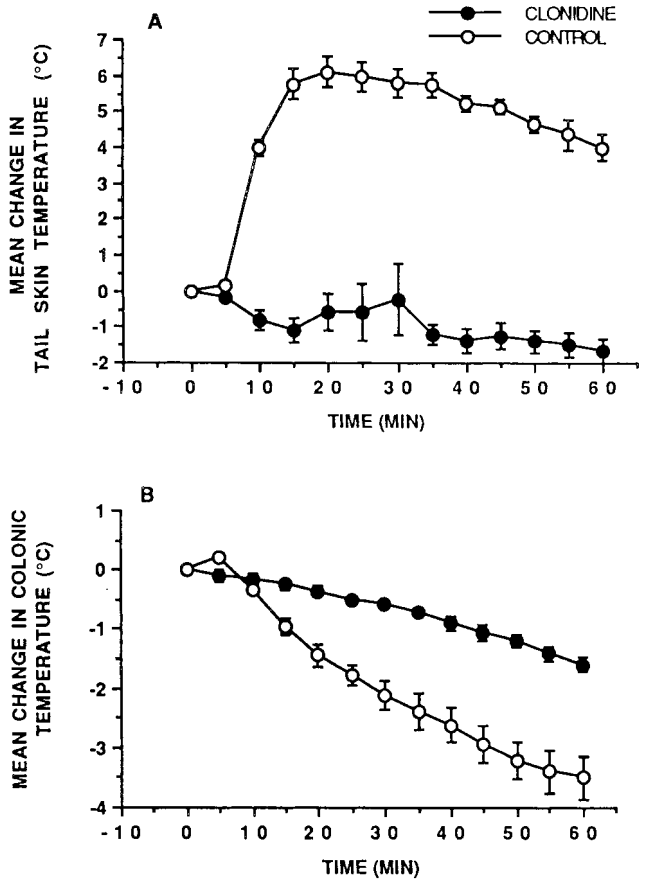

Figure 1. Effect of peripheral preadministration of clonidine (an α_2-adrenergic agonist, 0.5 mg/kg sc) on the mean changes in tail skin temperature (A) and colonic temperature (B) following administration of naloxone (1 mg/kg sc) to morphine-dependent female rats. Data are expressed as the mean ± 1 SE of six animals per group.

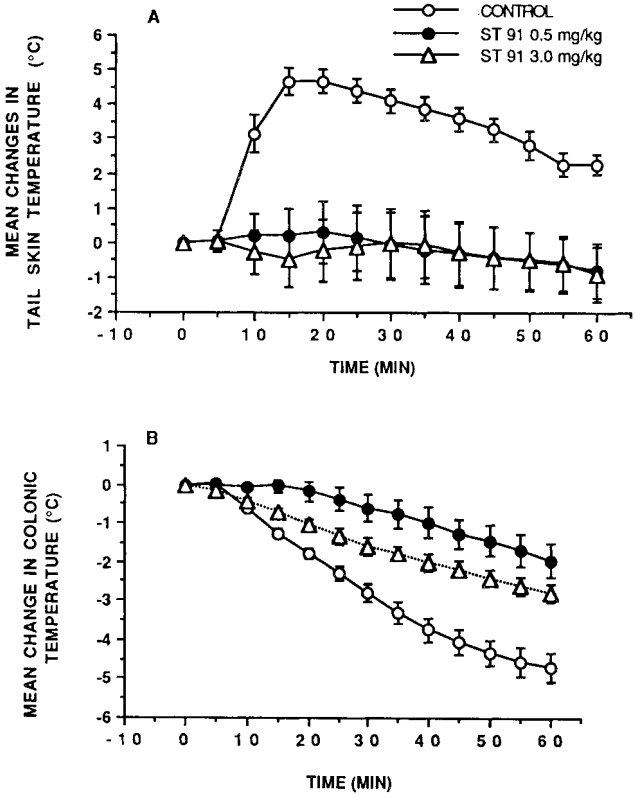

Figure 2. Effect of peripheral preadministration of an α_2-adrenergic agonist (ST-91, 0.5 and 3.0 mg/kg sc) on the mean change in tail skin temperature (A) and colonic temperature (B) following administration of naloxone (1 mg/kg sc) to morphine-dependent female rats. Data are expressed as the mean ± 1 SE of six animals per group.

which are mediated by sympathetic neurons of the central nervous system (11, 16, 17, 19). Recently, we have reported (11) that this response is antagonized by central administration of phentolamine and clonidine which implicates some central α_2-adrenergic component to this surge in TST observation in morphine-dependent rats. Although numerous investigators have demonstrated an enhanced central norepinephrine turnover associated with naloxone-precipitated withdrawal in morphine-dependent rats (2, 3, 5), the results presented in the present study would suggest that not only central but peripheral adrenergic events play a role in the surge in TST associated with morphine withdrawal.

The rat dissipates heat through increases in tail blood flow which is reflected by a change in tail skin temperature (20). This vasodilation can be manifested either by modulation of sympathetic adrenergic vasoconstrictor nerve activity and/or a separate active vasodilator system perhaps acting at arteriovenous shunts in the tail (21). The attenuated tail skin temperature response observed in adrenalectomized rats and propranolol-treated rats suggests that about 50% of the TST response may be due to a β-adrenergic mediated vasodilation of the tail vasculature. Propranolol may act to decrease vasodilation on TST by lowering metabolic rate, blocking vascular β-adrenergic receptors, and/or inhibiting peripheral catecholamine release (14, 22) in morphine-dependent rats. Similar mechanism(s) have been proposed for the attenuated TST response to isoproterenol in rats pretreated with propranolol (14). This later response to isoproterenal has been shown to be mediated via β_1-adrenoreceptors which act to increase metabolic rate (23). Thus, the actions of propranolol in the current study may have a similar effect in attenuating the rise in TST by altering metabolic rate in the morphine-dependent rat. Since removal of the predominant source of epinephrine by adrenalectomy also results in a 50% reduction in the surge in TST, a peripheral site of action is most likely for both effects.

The current study cannot address the precise role of the adrenals in this response. Several lines of evidence implicate glucocorticoids in various aspects of thermogenesis, such as prevention of heat loss via peripheral vasoconstriction, and the regulation of carbohydrate and lipid metabolism (24). Many or most of these effects are permissive in nature. Gwosdow and Besch (25) also have implicated a role for the adrenal gland in temperature responses associated with central opioid administration in the rat. The adrenal medullary hormones also can be implicated in the thermal change following administration of naloxone to morphine-de-

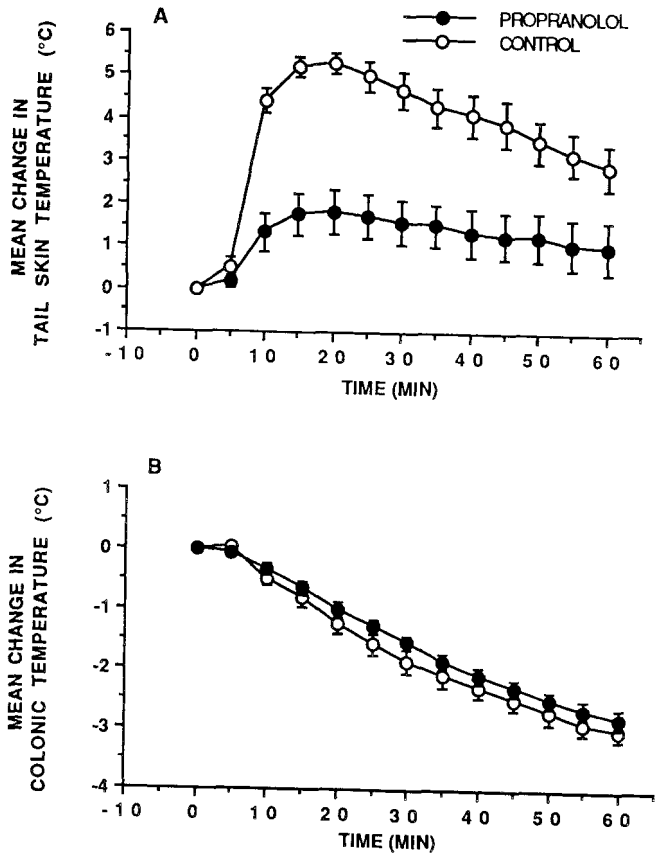

Figure 3. Effect of preadministration of *d,l*-propranolol (10 mg/kg sc) on the naloxone (1 mg/kg sc)-induced changes in mean tail skin (A) and colonic (B) temperatures in morphine-dependent female rats. Data are expressed as the mean ± 1 SE of six animals per group.

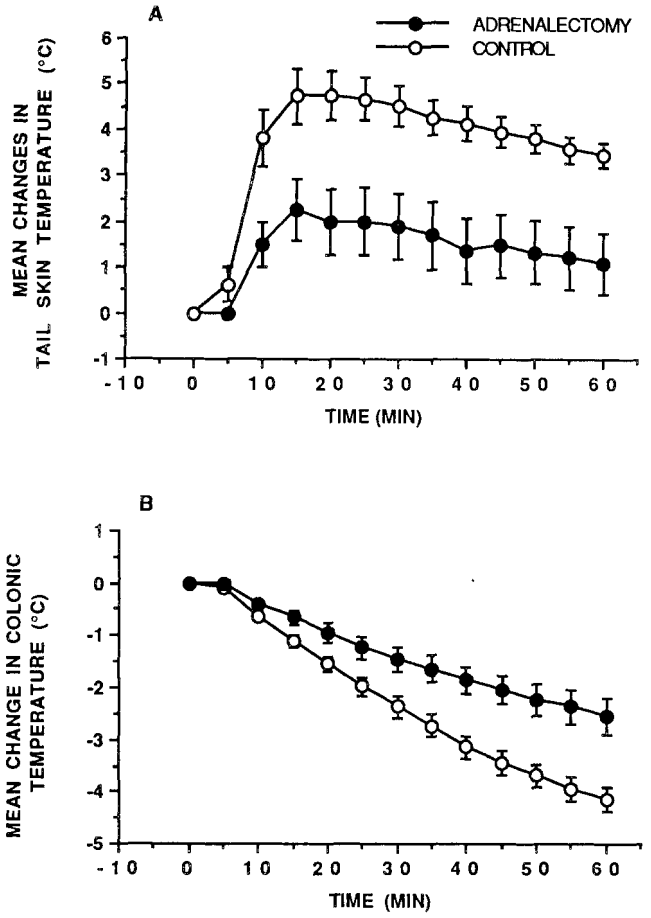

Figure 4. Effect of adrenalectomy (4 day) on the mean change in tail skin temperature (A) and the mean change in colonic temperature (B) following administration of naloxone (1 mg/kg sc) in morphine-dependent female rats. Data are expressed as the mean ± 1 SE of six animals per group.

pendent rats. A rise in adrenal medullary secreted epinephrine associated with administration of naloxone (4) may be the peripheral mediator responsible for the vasodilation which produces the observable rise in skin temperature during morphine withdrawal. Results from the α-adrenergic agonists to produce a vasoconstriction to lower this response would support the mechanism.

Treatment with the α_2-adrenergic agonists clonidine and ST-91 resulted in a total reduction of the TST surge in the morphine-dependent animals administered naloxone. Similar α-adrenergic agonists have been shown to be effective in ameliorating many of the symptoms of opiate withdrawal in humans (6) and in morphine-dependent rats (7). Tseng *et al.* (7) demonstrated that the effects of clonidine on withdrawal behavior were qualitatively similar when clonidine was administered centrally or peripherally to morphine-dependent rats. Central stimulation of α_2-adrenergic receptors by clonidine results in an inhibition of peripheral sympathetic activity (26, 27). This could be a mechanism responsible for the inhibitory effects of the α-agonists on the TST response in morphine-dependent rats. However, ST-91, which cannot cross the blood-brain barrier (12, 13), was as effective as clonidine when administered both peripherally and centrally, suggesting a peripheral as well as a central effect of both drugs. ST-91, an imidazoline derivative, is known to be 2.5 times more potent than clonidine as a peripheral vasoconstrictor, but has little central hypotensive effects when given systemically (13). The peripheral actions of the α-adrenergic agonists could be mediated by several mechanisms. It has been demonstrated that administration of clonidine results in a significant reduction of catecholamine secretion from the adrenal medulla (10, 28). Plasma epinephrine levels have been reported to be markedly increased during naloxone-precipitated withdrawal, an effect which is not apparent following adrenalectomy (4). This reduction of the outflow of adrenal epinephrine could contribute to an alteration in vascular tone and reduce the TST response. Additionally, systemic administration of these α-adrenergic agonists results in a transient elevation in blood pressure which results from a vasoconstriction via activation of postsynaptic α-agonists and clonidine enhances vasoconstrictor responses to sympathetic nerve stimulation (29). These direct effects of α-adrenergic agonists if active on the rat tail artery or the arteriovenous shunt of the tail would result in a reduced TST response. Another possible mechanism by which these drugs could attenuate the TST response is their hyperglycemic

effects (30). Hyperglycemia has been shown to reduce withdrawal-like behavior in morphine-dependent rats (31) and hypoglycemia can induce fluctuations in TST in rats (32). Although the precise mechanism(s) by which α_2-adrenergic agonists attenuate this temperature response in morphine-dependent rats is not completely understood, it appears that a peripheral action is as effective as a central action.

It has been suggested that low doses of morphine raise the set point of the central thermostats in the rat, resulting in a mild hyperthermia (33). All of the morphine-dependent rats tested displayed a mild hyperthermia. Although this hyperthermia has been suggested to be centrally mediated, the marked hypersecretion of norepinephrine associated with morphine treatment (4) may result in an increase in metabolism and/or a vasoconstriction contributing to in the elevated core temperature. Although the reduction in core temperature in morphine-dependent rats following administration of naloxone appears to have a central component, there is evidence for a peripheral component as well. We have previously demonstrated (19) that the degree of hypothermia is greater following systemic than central administration of naloxone. Lin *et al.* (34) attributed this fall in colonic temperature to both a decrease in metabolic heat production and cutaneous vasodilation. In the present study administration of α_1-adrenergic agonists and adrenalectomy but not propranolol treatment significantly attenuated the naloxone-induced hypothermia. Although the mechanism by which this attenuation occurs is not clear, the complete reduction in the TST response to naloxone was completely abolished only with α_2-adrenergic treatment. This would further suggest the possible differential mechanisms for the colonic and skin temperature changes associated with opioid withdrawal.

Collectively, the results obtained would suggest a role for the adrenal gland in mediating the skin temperature changes associated with withdrawal in the morphine-dependent rat. The observations presented raise the possibility that epinephrine-induced β_2-adrenoceptor-mediated vasodilation (35) coupled with a reduced α-adrenoceptor-mediated vasoconstriction may be responsible for the increase in tail skin temperature observed during morphine withdrawal. Clonidine administration affects both of these potential mechanisms, resulting in a total reduction of the TST response. Conversely, adrenalectomy and administration of propranolol may only affect one of these mechanisms, thus resulting in the partial reduction of the TST response. These results suggest that the rise in TST in our animal model is manifested at least in part by peripheral mechanisms.

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