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Potential role of the autonomic nervous system in the immunosuppressive effects of acute morphine administration

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Abstract

These studies investigated the role of the autonomic nervous system in mediating the immunosuppressive effect of morphine on blood lymphocyte proliferation in rats. To determine the contribution of the autonomic nervous system, rats were pretreated with the ganglionic blocker chlorisondamine (5 mg/kg) prior to morphine (7 mg/kg) administration. Ganglionic blockade with chlorisondamine completely antagonized the inhibitory actions of morphine, suggesting that intact ganglionic transmission was required for the inhibition to occur. Blockade of postganglionic parasympathetic neurotransmission with atropine methylbromide (1 mg/kg) or blockade of sympathetic neurotransmission with the α-adrenoceptor antagonist phentolamine (1 mg/kg) did not attenuate the suppressive effect of morphine. Blockade of β-adrenoceptors with propranolol (2.5 mg/kg) resulted in partial antagonism, but this action was not shared by the peripherally acting β-adrenoceptor antagonist nadolol (6 mg/kg). These results suggest that the inhibitory effect of morphine on blood lymphocyte proliferation may be mediated through activation of the autonomic nervous system; however, individual blockade of either the parasympathetic or sympathetic division of the autonomic nervous system was not sufficient to antagonize this immunosuppressive effect.

Keywords: Opioid; Immunity; Lymphocyte proliferation; Autonomic nervous system; Sympathetic division; Parasympathetic division; (Rat)

1. Introduction

It has been clearly established in animal models that exposure to opiate drugs alters a wide variety of immune parameters. For example, acute morphine administration in rats has been shown to suppress splenic natural killer cell cytolytic activity (Shavit et al., 1984; Bayer et al., 1990; Lysle et al., 1993) and inhibit splenic (Lysle et al., 1993) and peripheral blood (Bayer et al., 1990) lymphocyte proliferation. Peripheral blood lymphocytes appear to be particularly sensitive to the suppressive effect of morphine (Bayer et al., 1992). The suppression of both natural killer cell cytolytic activity and lymphocyte proliferation was found to be antagonized by pretreatment with naltrexone, demonstrating that these effects are mediated through opioid receptor activation (Weber and Pert, 1989; Bayer et al., 1990; Lysle et al., 1993). Although lymphocytes are thought to express opioid receptors (Sibinga and Goldstein, 1988; Garza et al., 1993; Makman, 1994), there is substantial evidence to support the hypothesis that many of the immunosuppressive actions of morphine are mediated indirectly. Shavit et al. (1986) were the first to report a central site of action for morphine-induced suppression of natural killer cell cytolytic activity. Similarly, we have reported that the inhibition of peripheral blood lymphocyte proliferation by morphine is mediated by stimulation of opioid receptors in the central nervous system (Hernandez et al., 1993).

The neuroendocrine system represents a potential pathway by which a centrally acting drug could alter peripheral immune cell activity. Morphine administration has been shown to activate the hypothalamic-pituitary-adrenal axis in rodents, leading to the release of a variety of pituitary and adrenal hormones (George and Way, 1955; Simonyi et al., 1988). Several of these hormones have been shown to directly modulate immune cell function and, therefore,

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could act as soluble mediators of the immunosuppressive effects of morphine (for review, see Bateman et al., 1989). While the immunosuppressive effects of chronic morphine treatment appear to be mediated by release of adrenal glucocorticoids (Bryant et al., 1991), the suppressive effects of acute morphine administration appear to be glucocorticoid independent. For example, we have reported that both adrenalectomy and hypophysectomy failed to antagonize the suppressive effect of acute morphine administration on blood lymphocyte proliferation in rats (Flores et al., 1994). These data demonstrate that activation of the hypothalamic-pituitary-adrenal axis does not significantly contribute to the suppressive effect of acute morphine administration on this immune parameter. In this report we considered the possibility that the autonomic nervous system may be involved in the suppressive effects of morphine on blood lymphocyte proliferation. There is a growing body of evidence which suggests that some of the effects of morphine on immune cell activity may be mediated through its effects on the autonomic nervous system. Opioid administration has been shown to alter the activity of both the sympathetic and the parasympathetic divisions of the autonomic nervous system. For example, systemic and central administration of morphine stimulated sympathetic outflow to the adrenal medulla and peripheral nerve endings, resulting in elevated plasma concentrations of epinephrine and norepinephrine in the rat (Conway et al., 1983). Similarly, intracisternal administration of β-endorphin and the μ-opioid selective agonist [D-Ala^2,*N*Me-Phe^4,Gly-ol^5]enkephalin (DAGO) resulted in substantial elevations of these circulating catecholamines (Appel et al., 1986). Further support of a possible autonomic nervous system-immune axis was the observation that lymphocytes possess β-adrenoceptors (Brodde et al., 1981) and that exposure to epinephrine, norepinephrine, or isoproterenol in vitro (Hadden et al., 1970) or norepinephrine in vivo (Felsner et al., 1992) suppressed lymphocyte proliferative responses to T cell mitogens. Finally, administration of α- and β-adrenoceptor antagonists prior to morphine in vivo has been reported to antagonize the inhibitory effect of morphine on splenic natural killer cell cytolytic activity (Carr et al., 1993) and splenic lymphocyte proliferation (Fecho et al., 1993), respectively. Since lymphocytes in the blood compartment are not directly innervated by the autonomic nervous system, these cells represent a unique regulatory target for the autonomic nervous system.

To test the hypothesis that morphine-induced suppression of blood lymphocyte activity is mediated by the autonomic nervous system, initial experiments were conducted in rats subjected to ganglionic blockade. Subsequent experiments focused on the individual contribution of the parasympathetic and sympathetic divisions of the autonomic nervous system. For these later studies, rats were pretreated with specific muscarinic receptor and adrenoceptor antagonists prior to morphine administration. Whenever possible, peripherally acting antagonists were used. Little is known about the central pathways utilized by morphine to alter immune cell activity, and the peripherally acting antagonists offer the advantage of leaving these pathways intact. The dose of each antagonist used in the immune studies was selected by conducting preliminary experiments in anesthetized rats. The effectiveness of each antagonist in blocking autonomic nervous system function was determined by its ability to inhibit the baroreceptor reflex.

2. Materials and methods

2.1. Animal care

Male Sprague-Dawley rats (200–300 g) were obtained from Harlan or Taconic. All rats were grouped housed (5/cage) with water and food (Purina Rat Chow) available ad libitum. The light cycle was automatically controlled (on 6 a.m.; off 6 p.m.) and the room temperature was regulated to maintain 23 ± 1°C. Animals were housed for 1–2 weeks to acclimate to these conditions before use in an experiment. Animals were handled on alternating days during the acclimation period to minimize handling stress on the day of the experiment. All experiments were conducted between 8:00 and 11:00 a.m.

2.2. Drug preparation and administration

Morphine sulfate (NIDA) and chlorisondamine (Ecolid, Ciba-Geigy) were dissolved in 0.9% saline. The following drugs were obtained from Sigma Chemical Company and dissolved in 0.9% saline: D,L-propranolol, L-phenylephrine, D,L-isoproterenol, atropine methyl-bromide, α-chloralose and urethane. Nadolol (Sigma) and phentolamine (Ciba-Geigy) were dissolved in sterile water. Injection volumes for drugs administered peripherally (either by s.c. or i.p. injection) did not exceed 0.25 ml.

2.3. Central microinjections

Surgery was performed on the rats one week prior to the experiments. Animals were anesthetized with Equithesin (1.1–1.5 ml/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). A guide cannula was directed to the third ventricle based on the coordinate system B as described (Pellegrino et al., 1979). With the incisor bar 5 mm above the interaural line, the coordinates for the injection site in the third ventricle were 2.8 mm posterior to bregma, 0 mm lateral to the midline, and 4.75 mm ventral to the surface of dura. A single 23-gauge stainless steel guide cannula (Biological Research Components, Roanoke, VA, USA) was placed through a hole drilled in the skull and fixed with dental acrylic and jeweler's screws. Each guide cannula was

fitted with a 28-gauge inert cannula to protect from debris and maintain patency.

One week after surgery, microinjections were carried out in awake and freely moving animals housed in individual cages. For microinjections into the third ventricle, chlorisondamine (5 μg/rat) was delivered in a total volume of 2 μl within 60 s through a 28-gauge internal injection cannula which extended 1 mm beyond the guide cannula. The injection cannula was connected to a piece of polyethylene tubing (PE-10), filled with saline or drug solution and attached to a 10 μl Hamilton syringe.

During the microinjections, the movement of an air bubble in the tubing provided a check that the solutions flowed freely. The injection cannula remained in place an additional 30 s to prevent the drug from being drawn back through the guide cannula. Following microinjections, animals were returned to group housing for the remainder of the experiment. Two hours later, animals were killed by decapitation and brains were dissected and placed in a 10% formalin solution. Sequential 75 μm coronal sections through the injection site were obtained using a freezing-stage microtome. Cannula site verification was executed by comparing the brain slices with the rat atlas as described (Pellegrino et al., 1979).

2.4. Analgesic measurements

The antinociceptive responses induced by morphine were assessed by the tail-flick test (D'Amour and Smith, 1941; Dewey and Harris, 1975). The tail-flick test was evoked by placing the rat's tail in a groove with a light-sensitive mechanism over an opening (2 cm) which housed a 100 W projection bulb. A switch started both the light and the timer. Once the tail was moved from the groove, the light striking the sensor would turn both the light and the timer off. The light intensity was controlled by a variable transformer set to give a pre-drug latency of 2.5–3.0 s. The test was terminated after 8.0 s to ensure that the unresponsive animal was not injured. Three consecutive trials were averaged for both pre- and post-drug responses. Data were expressed as percent of the maximal possible effect (% MPE) calculated from the following formula:

$$\%\,\mathrm{MPE} = \frac{\text{post-drug latency (s)} - \text{pre-drug latency (s)}}{\text{cutoff time} - \text{pre-drug latency}}$$

2.5. Lymphocyte proliferation

Rats were killed by decapitation and trunk blood was collected in heparinized tubes. Trunk blood was diluted 1 : 10 with RPMI 1640 (Gibco, Grand Island, NY, USA) containing 1% fetal calf serum (Biofluids, Rockville, MD, USA) and gentamicin (20 μg/ml, Gibco). The cell suspension (100 μl) was added to 96-well microtiter plates containing triplicate cultures (100 μl) of varying concentrations (1.0, 2.0, and 4.0 μg/culture) of the mitogen concanavalin A (Sigma). Following incubation for 72 h at 37°C with 5% CO_2, [^{3}H]methylthymidine (ICN Biomedicals, 6.7 Ci/mmol) was added (0.5 μCi/well) and cultures incubated for an additional 24 h. Labelled DNA was collected on glass fiber filters (Brandel) using a cell harvester (Brandel) and radioactivity determined by liquid scintillation spectrophotometry using a Taurus Beta Counter (Model 36010) with a counting efficiency for tritium of 30%.

The three concentrations of concanavalin A were selected to provide submaximal as well as maximal stimulation of proliferation. The maximal proliferative response occurred at either 2 μg or 4 μg concanavalin A/culture and was selected for data analysis because it provides the most accurate measure of the overall proliferative response of the lymphocytes. Selecting the maximal response does not alter the interpretation of the experimental data and a similar degree of suppression was observed with morphine at all mitogen concentrations, including submaximal concentrations.

2.6. Autonomic studies

Prior to the immune studies, preliminary experiments were conducted in anesthetized rats in order to select the appropriate dose of antagonist that would effectively block autonomic nervous system function. For each antagonist, several doses of drug were administered and the degree of receptor blockade was assessed by measuring the baroreceptor reflex. Only doses that produced effective blockade were included as data in Tables 1 through 3.

Animals were anesthetized with a combination of α-chloralose (60 mg/kg) and urethane (800 mg/kg). A cannula was placed in the femoral artery for blood pressure measurement and in the femoral vein for drug delivery. Changes in heart rate were recorded on an electrocardiogram.

Once baseline hemodynamic parameters were recorded, rats received an i.v. injection of phenylephrine or isoproterenol and the maximal change in blood pressure and heart rate was recorded (challenge 1). Following a 15 min recovery period, baseline blood pressure and heart rate values were again recorded (pre-drug) and rats received an i.p. injection of antagonist. New baseline parameters were recorded 30 min later (post-drug) and the phenylephrine or isoproterenol challenge was repeated (challenge 2).

2.7. Statistical analysis of data

Individual experiments were repeated at least 2 times. Differences among treatment groups were analyzed by analysis of variance and the Newman-Keuls test. The criterion for significance in all comparisons was $P < 0.05$.

3. Results

3.1. Chlorisondamine studies

In order to determine the role of the autonomic nervous system in mediating morphine-induced immunosuppression, initial experiments were performed in rats subjected to ganglionic blockade. Chlorisondamine was selected for these studies because it acts as competitive antagonist at nicotinic receptors in the ganglia (Sethi and Gulati, 1973) and has the advantage of being predominantly peripherally acting (Plummer et al., 1955). Since evidence of adequate ganglionic blockade is crucial for the interpretation of the subsequent immune studies, the degree of blockade produced by chlorisondamine was determined by examining its effect on the baroreceptor reflex. In anesthetized rats the pressor agent phenylephrine was administered intravenously to induce a sudden elevation of blood pressure which was followed by a compensatory decrease in heart rate. This compensatory decrease in heart rate following phenylephrine challenge should be abolished by ganglionic blockade.

The effect of chlorisondamine on the baroreceptor reflex is shown in Table 1. In the six animals tested, phenylephrine challenge 1 produced a mean increase in blood pressure of $+41 \pm 8$ mmHg and a mean decrease in heart rate of -62 ± 9 beats/min. Chlorisondamine treatment (5 mg/kg, i.p.) alone decreased blood pressure by -32 ± 5 mmHg and decreased heart rate by -97 ± 25 beats/min. Following chlorisondamine pretreatment, challenge 2 with phenylephrine produced an increase in blood pressure of $+36 \pm 11$ mmHg, which was similar to the increase observed pre-drug (+41 mmHg). In contrast to the lack of effect on the blood pressure response, the compensatory decrease in heart rate was completely abolished in chlorisondamine pretreated rats.

In order to determine whether ganglionic blockade would antagonize the inhibitory actions of morphine, rats received an i.p. injection of chlorisondamine 20 min prior to morphine administration. As previously reported, morphine administration (10 mg/kg, s.c.) resulted in an 80% suppression of blood lymphocyte proliferation (Fig. 1). Chlorisondamine pretreatment alone had no significant effect on the proliferative responses of the lymphocytes, as compared to saline-treated controls. In the presence of chlorisondamine, morphine administration had no inhibitory effect on blood lymphocyte proliferation.

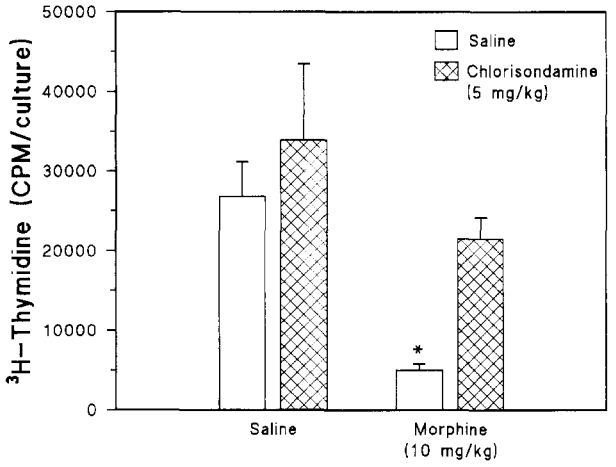

Fig. 1. Effect of chlorisondamine on morphine-induced suppression of blood lymphocyte proliferation. Rats received an i.p. injection of saline or chlorisondamine (5 mg/kg) followed 20 min later by a s.c. injection of saline or morphine sulfate (10 mg/kg). Animals were killed 2 h after their last injection. Proliferative responses of whole blood lymphocytes to concanavalin A were determined. Data are expressed as the mean ± S.E.M. cpm/culture at the maximal mitogen response for 5–7 animals per group. * Significantly different compared to double-injected, saline-treated controls.

Although chlorisondamine is considered to be a peripherally acting antagonist, it may cross the blood-brain barrier when administered at doses in the mg/kg range (Clarke, 1984). Therefore, to investigate the possibility that the actions of chlorisondamine were due to blockade of central nicotinic receptors, we administered chlorisondamine by i.c.v. injection. The results of centrally administered chlorisondamine are shown in Fig. 2. As observed with systemic administration, i.c.v. administration of chlorisondamine (5 μg/rat) had no effect on the proliferative response of blood lymphocytes. However, in contrast to

Table 1
Effect of chlorisondamine (CHL) on the baroreceptor reflex

Treatment ($n = 6$)	Blood pressure (BP)		Heart rate (HR)	
	mmHg	ΔBP	Beats/min	ΔHR
Pre-drug baseline	120 ± 5		411 ± 24	
Phenylephrine challenge 1	161 ± 7	+41 ± 8	349 ± 28	−62 ± 9
Pre-CHL baseline	113 ± 5		410 ± 23	
Post-CHL (5 mg/kg)	83 ± 3	−32 ± 5	312 ± 20	−97 ± 25
Phenylephrine challenge 2	118 ± 11	+36 ± 11	314 ± 16	+2 ± 4 [a]

Phenylephrine-induced alterations in blood pressure and heart rate were recorded in 6 anesthetized rats. Once baseline hemodynamic parameters were recorded, rats received an i.v. injection of phenylephrine (10 μg/kg, challenge 1). Following a 15 min recovery period, blood pressure and heart rate were recorded (Pre-CHL) and rats received an i.p. injection of CHL (5 mg/kg). New baseline parameters were recorded 30 min later (Post-CHL) and the phenylephrine challenge was repeated (challenge 2). Data are expressed as the mean ± S.E.M. [a] Significantly different compared to Δ HR of phenylephrine challenge 1.

systemic administration, central administration of chlorisondamine failed to antagonize morphine and a similar degree of inhibition of lymphocyte proliferation was observed in the presence or absence of chlorisondamine.

Studies investigating the specificity of chlorisondamine report the antagonist does not interact with peripheral muscarinic receptors, α-adrenoceptors, or β-adrenoceptors (Stone et al., 1958; Plummer et al., 1955). Our studies confirm the lack of interaction with peripheral α-adrenoceptors since chlorisondamine did not affect the pressor response to phenylephrine challenge (Table 1). To our knowledge, there have been no studies published on the potential interaction of the ganglionic blocker with opioid receptors. To test this possibility, the effect of chlorisondamine on opioid-receptor mediated analgesia was studied. Analgesia was measured by the tail-flick assay (see Section Section 2). As seen in Fig. 3, on day 1 morphine produced maximal analgesia when administered by s.c. injection at a dose of 2 mg/kg. On the following day (day 2), chlorisondamine (5 mg/kg, i.p.) was administered to the same group of rats and produced no analgesia. When morphine (2 mg/kg, s.c.) was administered to these chlorisondamine pretreated animals, maximal analgesia was again observed. These data demonstrate that chlorisondamine alone does not produce analgesia and has no effect on opioid-induced analgesic responses.

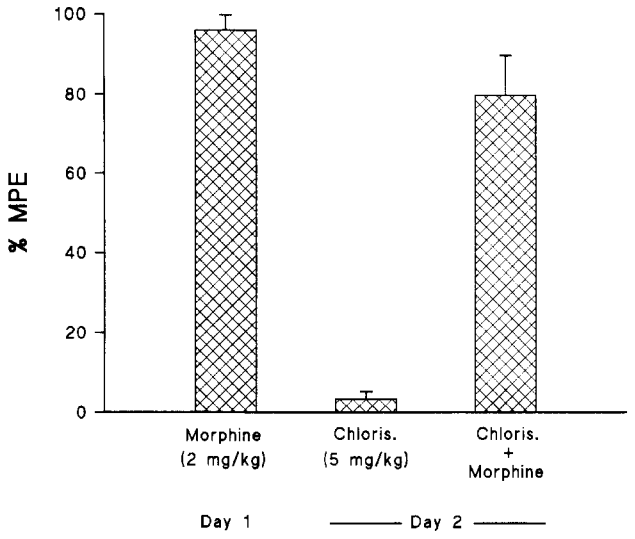

Fig. 3. Effect of chlorisondamine on morphine-induced analgesia. On day 1 of the experiment, rats received a s.c. injection of morphine sulfate (2 mg/kg) and tail-flick latencies were recorded 30 min later. On day 2 of the experiment, the same rats received an i.p. injection of chlorisondamine (5 mg/kg) followed 30 min later by a s.c. injection of morphine sulfate (2 mg/kg). Tail-flick latencies were recorded prior to chlorisondamine administration, 30 min post chlorisondamine, and 30 min post morphine. Data are expressed as the mean ± S.E.M. % maximal possible effect (% MPE) for 3–4 animals.

3.2. Atropine methylbromide studies

To study the involvement of the parasympathetic division of the autonomic nervous system, animals were pretreated with the peripherally acting muscarinic receptor antagonist atropine methylbromide. In order to select an appropriate dose of antagonist, the effect of atropine methylbromide on pupillary dilation was studied. Regulation of pupil size is controlled by postganglionic parasympathetic neurons and blockade of muscarinic receptors with atropine results in dilated pupils (Weiner, 1985). In preliminary studies it was found that a dose of 1 mg/kg of atropine methylbromide was sufficient to produce a complete, long-lasting pupillary dilation in the rat (data not shown). In order to determine whether muscarinic blockade would antagonize the inhibitory actions of morphine on blood lymphocyte proliferation, rats were treated with atropine methylbromide (1 mg/kg, i.p.) 20 min prior to morphine (10 mg/kg, s.c.) administration. As seen in Fig. 4, atropine pretreatment alone had no effect on the proliferative responses of the lymphocytes when compared to saline-treated controls. Morphine maximally suppressed blood lymphocyte proliferation both alone and in the presence of atropine methylbromide.

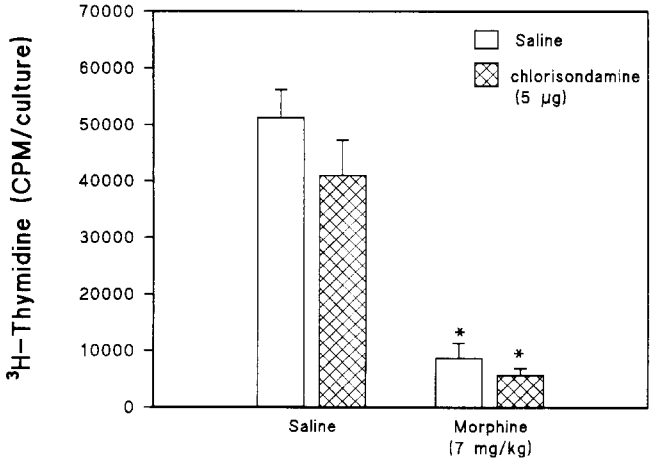

Fig. 2. Effect of centrally administered chlorisondamine on morphine-induced suppression of blood lymphocyte proliferation. Rats received an i.c.v. injection of saline or chlorisondamine (5 μg/rat) followed 30 min later by a s.c. injection of saline or morphine sulfate (7 mg/kg). Animals were killed 2 h after their last injection. Proliferative responses of whole blood lymphocytes to concanavalin A were determined. Data are expressed as the mean ± S.E.M. cpm/culture at the maximal mitogen response for 5–6 animals per group. * Significantly different compared to double-injected, saline-treated controls.

3.3. Phentolamine studies

In order to identify a dose of phentolamine that was sufficient to block peripheral α-adrenoceptors, the effect of phentolamine on phenylephrine-induced elevations in blood pressure was studied. These studies used anesthetized rats and an experimental design similar to that described for studying ganglionic receptor blockade. However, unlike

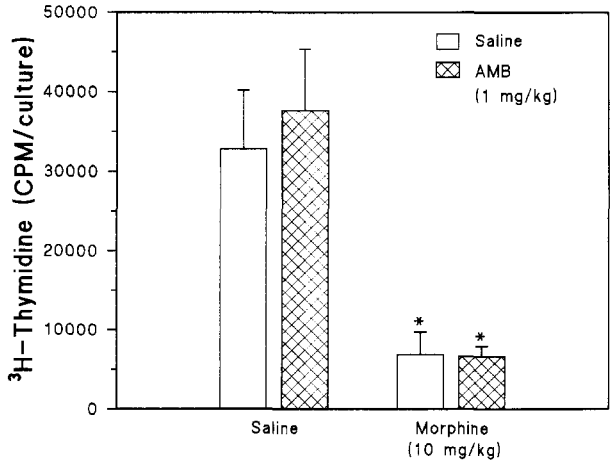

Fig. 4. Suppression of blood lymphocyte proliferation by morphine in rats pretreated with atropine methylbromide. Rats received an i.p. injection of saline or atropine methylbromide (1 mg/kg) followed 20 min later by a s.c. injection of saline or morphine sulfate (10 mg/kg). Animals were killed 2 h after their last injection. Proliferative responses of whole blood lymphocytes to concanavalin A were determined. Data are expressed as the mean ± S.E.M. cpm/culture at the maximal mitogen response for 4–6 animals per group. * Significantly different compared to double-injected, saline-treated controls.

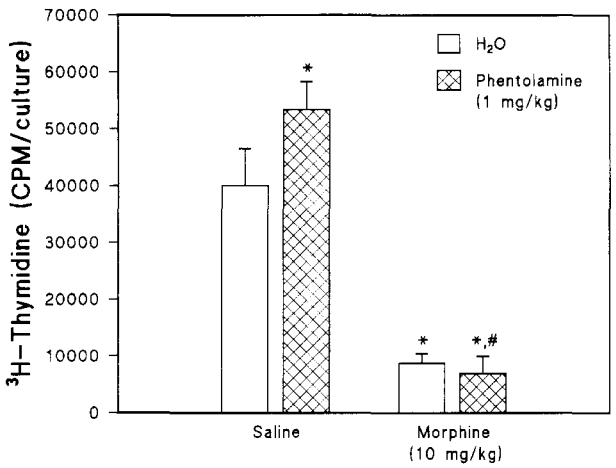

Fig. 5. Suppression of blood lymphocyte proliferation by morphine in rats pretreated with phentolamine. Animals received an i.p. injection of water or phentolamine (1 mg/kg) followed 20 min later by a s.c. injection of saline or morphine sulfate (10 mg/kg). Animals were killed 2 h after their last injection. Proliferative responses of whole blood lymphocytes to concanavalin A were determined. Data are expressed as the mean ± S.E.M. cpm/culture for 5–6 animals per group. * Significantly different compared to water/saline-treated controls.

ganglionic blockers which had no direct effect on phenylephrine-induced elevations in blood pressure, the ability of phentolamine to directly antagonize this α-mediated increase in blood pressure was the measure of effective blockade.

The effect of phentolamine on phenylephrine-induced alterations of blood pressure and heart rate is shown in Table 2. As demonstrated previously, an i.v. injection of phenylephrine (10 μg/kg; challenge 1) increased blood pressure by +30 ± 4 mmHg and caused a reflex decrease in heart rate of −35 ± 12 beats/min. Phentolamine treatment (1 mg/kg, i.p.) alone decreased blood pressure by −12 ± 5 mmHg and decreased heart rate by −15 ± 6 beats/min. In the presence of phentolamine, the pressor response to phenylephrine was significantly attenuated (+4 ± 4 mmHg; challenge 2). In the absence of a significant pressor response, the baroreceptor reflex was blunted and the compensatory decrease in heart rate was also attenuated (−4 ± 2 beats/min). It is concluded that this dose of phentolamine is sufficient to block peripheral α-adrenoceptors.

To determine whether α-adrenoceptor blockade with phentolamine could antagonize the inhibitory actions of morphine on blood lymphocyte activity, rats were treated with phentolamine (1 mg/kg, i.p.) 20 min prior to morphine (10 mg/kg, s.c.) administration. As seen in Fig. 5, phentolamine pretreatment alone slightly enhanced the proliferative responses of blood lymphocytes when compared to saline-treated controls. In the absence or presence of phentolamine, morphine inhibited lymphocyte proliferation by 78% and 87%, respectively.

Table 2
Effect of phentolamine on phenylephrine-induced alterations of blood pressure and heart rate

Treatment (n = 3)	Blood pressure (BP)		Heart rate (HR)	
	mmHg	ΔBP	Beats/min	ΔHR
Pre-drug baseline	115 ± 6		391 ± 17	
Phenylephrine challenge 1	145 ± 7	+30 ± 4	356 ± 16	−35 ± 12
Pre-phentolamine baseline	111 ± 6		379 ± 19	
Post-phentolamine (1 mg/kg)	100 ± 3	−12 ± 5	364 ± 18	−15 ± 6
Phenylephrine challenge 2	104 ± 7	+4 ± 4 [a]	360 ± 17	−4 ± 2

Phenylephrine-induced alterations in blood pressure and heart rate were recorded in 3 anesthetized rats. Once baseline hemodynamic parameters were recorded, rats received an i.v. injection of phenylephrine (10 μg/kg, challenge 1). Following a 15 min recovery period, blood pressure and heart rate were recorded (Pre-phentolamine) and rats received an i.p. injection of phentolamine (1 mg/kg). New baseline parameters were recorded 30 min later (Post-phentolamine) and the phenylephrine challenge was repeated (challenge 2). Data are expressed as the mean ± S.E.M. [a] Significantly different compared to Δ BP of phenylephrine challenge 1.

3.4. Propranolol studies

The following studies were designed to investigate the potential role of peripheral β-adrenoceptor activation in mediating the suppressive effects of morphine on blood lymphocyte activity. The nonselective β_1- and β_2-adrenoceptor antagonist propranolol was selected for initial studies. This antagonist has equal affinity for β_1- and β_2-adrenoceptors (Bieth et al., 1980), but is highly lipid soluble (Frishman, 1979) and crosses the blood-brain barrier easily, increasing the potential for blocking central β-adrenoceptors. In order to identify a dose of propranolol that was sufficient to block peripheral β-adrenoceptors, the effect of propranolol on isoproterenol-induced alterations of blood pressure and heart rate was studied.

The effect of propranolol on isoproterenol-induced alterations in blood pressure and heart rate is shown in Table 3. Isoproterenol challenge 1 (5 μg/kg, i.v.) decreased blood pressure by -52 ± 3 mmHg and increased heart rate by $+27 \pm 8$ beats/min. Propranolol pretreatment (2.5 mg/kg, i.p.) resulted in a fall in blood pressure of -20 ± 7 mmHg and decrease in heart rate of 112 ± 12 beats/min. When the isoproterenol challenge was repeated in the presence of propranolol, 67% of the blood pressure response was blocked (-52 ± 3 vs. -17 ± 4 mmHg). In contrast to the blood pressure response, the isoproterenol-induced increase in heart rate was not attenuated by propranolol pretreatment. It is concluded that at this dose of 2.5 mg/kg, a partial blockade of β-adrenoceptors was achieved.

The effect of propranolol pretreatment on morphine-induced suppression of blood lymphocyte activity is shown in Fig. 6. In this study rats were pretreated with propranolol (2.5 mg/kg, i.p.) 20 min prior to morphine (10 mg/kg, s.c.) administration. Propranolol pretreatment alone at this dose had no significant effect on the proliferative response of lymphocytes when compared to saline-treated controls. While morphine administration resulted in an 85% suppression in the saline-pretreated rats, only a 55% suppression was observed in the propranolol-pretreated group. This partial antagonism was statistically significant when the responses of the two morphine- treated groups were compared. This partial antagonism was also observed when lower doses of morphine (6 mg/kg, s.c.) were administered to propranolol (2.5 mg/kg, i.p.) pretreated rats (data not shown). Since a partial blockade was observed in both the autonomic and immune studies with 2.5 mg/kg propranolol, a higher dose (5 mg/kg, i.p.) was selected for subsequent studies with the hope of producing a more substantial blockade. Unfortunately, immune studies revealed that this higher dose of propranolol produced a significant suppression of lymphocyte proliferation on its own (data not shown).

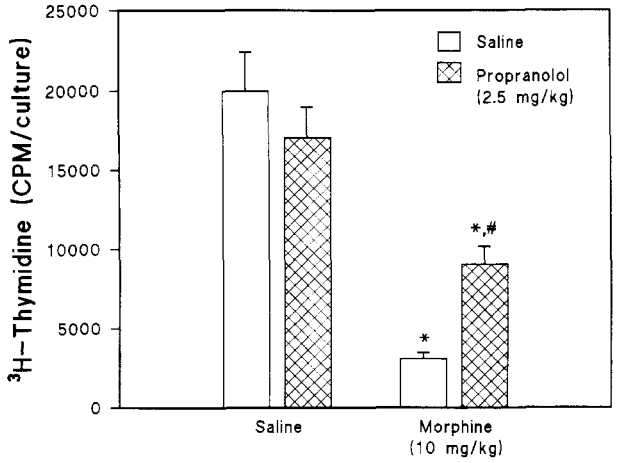

Fig. 6. Suppression of blood lymphocyte proliferation by morphine in rats pretreated with propranolol. Animals received an i.p. injection of saline or propranolol (2.5 mg/kg) followed 20 min later by a s.c. injection of saline or morphine sulfate (10 mg/kg). Animals were killed 2 h after their last injection. Proliferative responses of whole blood lymphocytes to concanavalin A were determined. Data are expressed as the mean ± S.E.M. cpm/culture at the maximal mitogen response for 5–6 animals per group. * Significantly different compared to double-injected, saline-treated controls. # Significantly different compared to morphine-treated group.

3.5. Nadolol studies

Nadolol, a nonselective β-adrenoceptor antagonist, is less lipid soluble than propranolol and has a reduced capacity to cross the blood-brain barrier (Rudd and Blaschke, 1985). In order to characterize the peripheral

Table 3
Effect of propranolol on isoproterenol-induced alterations of blood pressure and heart rate

Treatment ($n = 3$)	Blood pressure (BP)		Heart rate (HR)	
	mmHg	ΔBP	Beats/min	ΔHR
Pre-drug baseline	129 ± 6		395 ± 34	
Isoproterenol challenge 1	77 ± 7	−52 ± 3	422 ± 30	+27 ± 8
Pre-propranolol baseline	117 ± 8		373 ± 39	
Post-propranolol (2.5 mg/kg)	97 ± 11	−20 ± 7	261 ± 28	−112 ± 12
Isoproterenol challenge 2	80 ± 12	+17 ± 4	267 ± 36	+36 ± 11

Isoproterenol-induced alterations in blood pressure and heart rate were recorded in 3 anesthetized rats. Once baseline hemodynamic parameters were recorded, rats received an i.v. injection of isoproterenol (5 μg/kg, challenge 1). Following a 15 min recovery period, blood pressure and heart rate were recorded (Pre-propranolol) and rats received an i.p. injection of propranolol (2.5 mg/kg). New baseline parameters were recorded 30 min later (Post-propranolol) and the isoproterenol challenge was repeated (challenge 2). Data are expressed as the mean ± S.E.M.

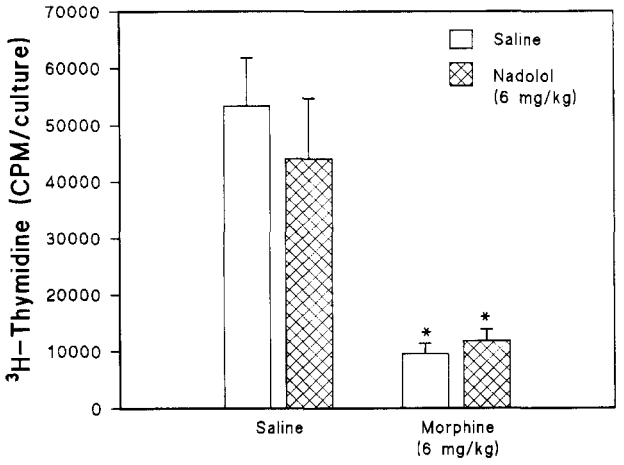

Fig. 7. Suppression of blood lymphocyte proliferation by morphine in rats pretreated with nadolol. Animals received an i.p. injection of water or nadolol (6 mg/kg) followed 60 min later by a s.c. injection of saline or morphine sulfate (6 mg/kg). Animals were killed 2 h after their last injection. Proliferative responses of whole blood lymphocytes to concanavalin A were determined. Data are expressed as the mean ± S.E.M. cpm/culture at the maximal mitogen response for 6 animals per group. * Significantly different compared to double-injected controls.

β-adrenoceptor blocking properties of nadolol in the rat, the effect of the drug on isoproterenol-induced alterations of blood pressure and heart rate were studied as described above for propranolol. Nadolol treatment (6 mg/kg, i.p.) blocked approximately 35% of the blood pressure response (-52 ± 10 vs. -34 ± 9 mmHg) and 50% of the heart rate response ($+44 \pm 18$ vs. $+20 \pm 5$ beats/min) to isoproterenol challenge (data not shown). It is concluded that at this dose, a partial blockade of β-adrenoceptor was achieved.

The effect of nadolol pretreatment on morphine-induced suppression of blood lymphocyte activity is shown in Fig. 7. Nadolol pretreatment (6 mg/kg, i.p.) alone had no effect on the proliferative response of the lymphocytes when compared to saline-treated controls. In the presence or absence of nadolol, morphine administration resulted in maximal suppression of lymphocyte proliferation. Similar results were obtained with the peripherally acting β_1-adrenoceptor selective antagonist atenolol. When administered by i.p. injection at a dose of 15 mg/kg, atenolol alone had no effect on the proliferative response of lymphocytes and failed to effectively antagonize the inhibitory action of morphine (data not shown).

4. Discussion

We have previously reported that the suppressive effect of morphine on blood lymphocyte activity is centrally mediated (Hernandez et al., 1993), independent of the hypothalamic-pituitary-adrenal axis (Flores et al., 1994), and not mediated by alterations in circulating lymphocyte numbers or subsets (Flores et al., 1995). The present studies were initiated to determine the possible role of the autonomic nervous system in the suppressive effect of morphine on circulating blood lymphocytes. In the first series of experiments, the effect of ganglionic blockade on the inhibitory actions of morphine was studied. Subsequent studies employed specific receptor antagonists to examine the contribution of postganglionic muscarinic receptors and adrenoceptors to the inhibitory actions of morphine. For each antagonist used, experiments were designed to assess the degree of receptor blockade achieved.

The ganglionic blocker chlorisondamine was selected for the initial studies. Chlorisondamine, a bisquaternary tetrachloroisoindoline, differs from mecamylamine and hexamethonium in several important regards. Chlorisondamine is a much more potent ganglionic blocker and has a much longer duration of action (Plummer et al., 1955; Schneider and Moore, 1955). In rats, chlorisondamine has been shown to be superior to hexamethonium as a ganglionic blocker (Abdel-Rahman, 1989). Since the initial description of chlorisondamine by Plummer et al. (1955), several investigators have characterized this drug in regard to its potency, duration of action, and specificity for the nicotinic receptor. Physiological studies in vivo or pharmacological studies in vitro in isolated muscle preparations suggest that chlorisondamine does not interact with peripheral adrenoceptors, muscarinic receptors, or histamine receptors (Plummer et al., 1955; Stone et al., 1958). In addition to these studies, our data demonstrated that chlorisondamine does not appear to interact with opioid receptors since the drug did not attenuate opioid-induced analgesia (Fig. 3).

Based on the studies by Abdel-Rahman (1989), we selected the dose of 5 mg/kg chlorisondamine to produce ganglionic blockade in rats. As previously reported, this dose produced complete ganglionic blockade (Table 1). This dose also effectively blocked the inhibitory actions of morphine on blood lymphocyte activity (Fig. 1), suggesting that intact ganglionic transmission is required for the inhibition to occur. Since it has been suggested that, at high doses, chlorisondamine can cross the blood-brain barrier (Clarke, 1984), we considered the possibility that blockade of central nicotinic receptors was the mechanism by which chlorisondamine antagonized morphine. To investigate this possibility, we administered chlorisondamine centrally. Microinjection of chlorisondamine into the fourth ventricle failed to antagonize peripherally administered morphine (Fig. 2). Failure of centrally administered chlorisondamine to antagonize morphine could not be attributed to insufficient amount of drug since the dose of chlorisondamine selected for these studies (5 μg/rat) has been shown to effectively block the effects of peripherally administered nicotine on locomoter activity in rats (Clarke, 1984). We conclude, therefore, that the actions of systemically administered chlorisondamine are mediated by blockade of ganglionic nicotinic receptors.

A pharmacological approach was utilized in an attempt

to identify the postganglionic mechanisms involved in morphine-induced suppression of blood lymphocyte activity. Blockade of postganglionic parasympathetic neurotransmission with atropine methylbromide did not attenuate the suppressive effects of morphine on blood lymphocyte activity. Similarly, blockade of postganglionic sympathetic neurotransmission with the α-adrenoceptor antagonist phentolamine did not antagonize the suppression of lymphocyte activity observed following morphine treatment. Blockade of β-adrenoceptors with propranolol resulted in a partial antagonism, but this activity was not shared by the peripherally acting antagonists nadolol or atenolol. The differential ability of these two drugs to antagonize morphine is not due to a differential degree of peripheral receptor blockade since autonomic studies demonstrated that these two compounds produce a similar degree of receptor blockade. Since propranolol has been shown to block 5-HT (5-hydroxytryptamine, serotonin) receptors in addition to β-adrenoceptors (Tsuchihashi et al., 1990), a property not shared by nadolol, we considered the possibility that the partial antagonism by propranolol was a result of 5-HT receptor blockade. Pretreatment of animals with the nonselective 5-HT receptor antagonist methysergide (Bradley et al., 1986), however, had no effect on morphine-induced suppression of blood lymphocyte proliferation (data not shown). It is unlikely, therefore, that the partial antagonism with propranolol is a result of 5-HT receptor blockade. A more likely hypothesis is that the partial antagonism is due to blockade of central β-adrenoceptors since propranolol is a highly lipophilic compound and enters the central nervous system easily (Frishman, 1979; Rudd and Blaschke, 1985). The more hydrophilic compounds, nadolol and atenolol, have a reduced capacity to enter the central nervous system (Sethi and Gulati, 1973). Since little is known about the central pathways utilized by morphine to inhibit peripheral immune cell activity, one must consider the potential requirement for β-adrenoceptor activation.

In summary, the ability of chlorisondamine to completely antagonize the actions of morphine demonstrates that the signal is transmitted from the central nervous system through the ganglia. The postganglionic mechanism by which morphine alters circulating immune cell activity, however, remains elusive. Our data strongly suggest that the individual blockade of peripheral muscarinic receptors, α-adrenoceptors or β-adrenoceptors does not attenuate the suppressive actions of morphine. These data are in agreement with those of Fecho et al. (1993) who also reported pretreatment of rats with β-adrenoceptor antagonists failed to block the suppressive effect of morphine on blood lymphocyte responses to mitogens. The participation of α- or β-adrenoceptors in mediating the immunosuppressive action of morphine cannot be ruled out definitively due to the limitations inherent to this type of pharmacological approach. Limitations such as inadequate degree of peripheral receptor blockade, blockade of central as well as peripheral receptors, and inhibition of lymphocyte activity by the antagonist alone may all contribute to the difficulty of interpreting data from pharmacological studies such as these.

One interpretation of the present findings is that the activity of noncholinergic, nonadrenergic postganglionic nerves is responsible for the suppression of lymphocyte proliferation observed following morphine treatment. This mechanism would be independent of peripheral muscarinic receptor or adrenoceptor activation and would be consistent with the inability of the peripheral receptor blockers to antagonize morphine. As an alternative hypothesis, one could propose that the suppression of lymphocyte activity observed following morphine treatment is mediated by the release of neuropeptides from either postganglionic parasympathetic or sympathetic neurons. Neuroactive peptides have been identified in most postganglionic nerves. For example, vasoactive intestinal peptide is released with acetylcholine from parasympathetic nerves. Vasoactive intestinal peptide has been shown to increase glandular blood flow and augment salivation (Cooper et al., 1991). Neuropeptide Y is found in many sympathetic neurons and has been shown to sensitize smooth muscle target cells to adrenergic signals (Cooper et al., 1991). Somatostatin is also co-released from many sympathetic neurons. In support of a potential immunomodulatory role for the neuropeptides is the demonstration that lymphocytes possess specific, high affinity binding sites for substance P, vasoactive intestinal peptide, and somatostatin (Payan and Goetzl, 1985). Additionally, these peptides have been shown to enhance and inhibit lymphocyte activity in vitro at concentrations in the nM range (Payan and Goetzl, 1985). Substance P appears to be stimulatory in nature and has been reported to enhance the proliferative responses of human T lymphocytes, stimulate chemotaxis, and stimulate H_2O_2 production from macrophages. Vasoactive intestinal peptide activates adenylate cyclase in human lymphocytes and inhibits the proliferative responses of murine lymphoblasts. Interestingly, somatostatin inhibits the proliferative responses of human T lymphocytes in vitro at concentrations as low as 10^{-12} M (Payan and Goetzl, 1985). These neuropeptides, therefore, have the potential to be novel immunomodulatory agents.

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