Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 523–529, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0155-B

Atipamezole, an Alpha$_2$ Antagonist, Augments Opiate-Induced Muscle Rigidity in the Rat

MATTHEW B. WEINGER[1] AND JULIE MIRIAM BEDNARCZYK

Department of Anesthesiology, University of California, San Diego, CA and
Opiate Neuropharmacology Research Laboratory, San Diego Veterans Affairs Medical Center, San Diego, CA

Received 27 April 1993

WEINGER, M. B. AND J. M. BEDNARCZYK. *Atipamezole, an alpha$_2$ antagonist, augments opiate-induced muscle rigidity in the rat.* PHARMACOL BIOCHEM BEHAV **49**(3) 523–529, 1994.—Atipamezole is a new, highly selective alpha$_2$-adrenoceptor antagonist currently undergoing clinical trials as an antagonist for dexmedetomidine, a potent alpha$_2$ agonist with sedative and analgesic properties. It has previously been demonstrated that dexmedetomidine, acting at central alpha$_2$ adrenoceptors, antagonizes opiate-induced muscle rigidity. However, the role of endogenous alpha$_2$-adrenergic systems in opiate-induced rigidity remains to be elucidated. The present study was designed to assess the effects of atipamezole on basal muscle tone and on alfentanil-induced muscle rigidity in the rat. Muscle tone was measured using gastrocnemius electromyography (EMG). After a 15-min baseline, saline or atipamezole (0.3 or 1.0 mg/kg) was administered, and 10 min later, saline or alfentanil (50, 150, or 300 μg/kg) was injected subcutaneously. Data were collected for an additional 60 min. Atipamezole (1.0 mg/kg) pretreatment (in the absence of alfentanil) produced a small increase in tonic EMG activity when compared with saline pretreatment. After saline pretreatment, significant muscle rigidity occurred in the two highest alfentanil dose groups. Atipamezole (0.3 and 1.0 mg/kg) augmented alfentanil-induced muscle rigidity. The ability of the alpha$_2$ antagonist to potentiate both basal muscle tone and alfentanil-induced rigidity suggests that endogenous adrenergic activity and/or direct alpha$_2$-adrenoceptor interaction with opioid receptors mediate opiate-induced muscle rigidity. These findings may be of clinical as well as basic neuropharmacological interest.

Muscle tone Alpha$_2$-adrenergic antagonist Atipamezole Opiate Muscle rigidity Alfentanil Dexmedetomidine Anesthesia

ALPHA$_2$-ADRENERGIC drugs have elicited increasing clinical interest because of their unique pharmacological profile. New alpha$_2$ agonists, such as dexmedetomidine (DEX), produce analgesia (36) and sedation (40,41) with minimal ventilatory depression (6,32). DEX also reverses or prevents opiate-induced muscle rigidity, a potentially troublesome side effect of high-dose opiate anesthesia (51). One important potential clinical benefit of the alpha$_2$ agonists is the existence of highly selective and specific antagonists that readily reverse the agonists' sedative effects (24). Atipamezole (ATIP) is a centrally acting alpha$_2$-adrenergic antagonist that has been shown to selectively bind to alpha$_2$ adrenoceptors with little or no effect on other neurochemical receptors (48). ATIP dose-dependently antagonizes the behavioral, sedative, and neurohumeral effects of alpha$_2$ agonists in both animals (39) and humans (2,24).

The combined use of the alpha$_2$ agonist DEX and an opiate for total intravenous anesthesia, and its subsequent reversal with ATIP and an opiate antagonist, has already been described in the veterinary literature (19). Because of the potential value of this anesthetic technique, similar studies are likely in human subjects. However, reversal of DEX sedation in human volunteers with a large dose of ATIP resulted in overshoot in plasma norepinephrine levels of 50% above basal levels (24). Additionally, ATIP alone dose-dependently increased salivation, blood pressure, and plasma noradrenaline levels in humans. The highest ATIP doses produced hypervigilance, nervousness, tremor, shivering, and sweating, effects opposite of those seen with alpha$_2$ agonists (25). These findings suggest that endogenous alpha$_2$-adrenergic systems may play a role in these behaviors.

The alpha$_2$ agonist, DEX, antagonizes muscle rigidity produced by alfentanil (ALF), a potent opiate agonist in the rat (51). The concommitant administration of idazoxan, an alpha$_2$ antagonist, reversed DEX's effects (51). These data have led ourselves (51) and others (28,46) to the hypothesis

[1] Requests for reprints should be addressed to Matthew B. Weinger, M.D., Veterans Affairs Medical Center (125), 3350 La Jolla Village Drive, San Diego, CA 92161-5085.

that alpha$_2$-adrenergic systems mediate opiate-induced muscle rigidity. In preliminary studies, it was observed that administration of alpha$_2$ antagonists not only blocked alpha$_2$ agonist effects, but, in and of themselves, appeared to augment opiate rigidity. Substantiation of this observation would lend additional support to a role for endogenous noradrenergic systems in the increased muscle tone that occurs after high-dose opiate administration.

METHOD

Animals

The subjects were 92 male albino Wistar rats (Harlan Laboratories, Chicago, IL) weighing 243–424 g. They were housed two–three per cage in a temperature-controlled room, with food and water available continuously. Each rat was acclimated to the experimental apparatus, a cylindrical olding device, in three 1-h sessions prior to the experiment. All experiments were performed between 1200 and 1700 h. This study was approved by the San Diego VA Medical Center's Animal Care Committee and conformed with local and national guidelines for the care and use of experimental animals.

Drugs

The drugs used were alfentanil hydrochloride (ALF; Janssen Pharmaceutica, Piscataway, NJ) and atipamezole hydrochloride (ATIP; Orion Corporation, Turku, Finland). The drugs, obtained as powders, were dissolved in 0.9% sterile physiological saline and injected subcutaneously in a volume of 1 ml/kg. The doses of ATIP were 0.3 and 1.0 mg/kg and the control group was given identical volumes of saline. ALF was administered at doses of 50, 150, and 300 μg/kg. These ALF doses had been previously shown to produce minimal, mild, or moderate (respectively) muscle rigidity in the model used in the present study (57). All injections were made in a blinded fashion, and animals were randomly assigned to treatment groups.

Electromyographic Measurements

Rats were placed in cylindrical holding devices, which allowed free movement of the extremities and easy access to injection and recording sites. Hindlimb electromyographic (EMG) activity was recorded as described previously (50–52). Briefly, two Grass monopolar platinum recording electrodes were placed percutaneously into the left gastrocnemius muscle, and a third (ground) electrode was inserted subcutaneously into the right leg. Leads were secured in such a way as to permit unimpeded joint mobility, and each rat was placed in a separate soundproof box (Coulbourn Instrument Co.). Muscle potentials were differentially amplified 100 times and band-pass filtered from 10 Hz to 3 kHz (Grass P511K). The amplified EMG signal was viewed on an oscilloscope (Tektronics 7633) and a permanent record was generated using a Grass 4-channel strip-chart recorder. The signal was processed using a root-mean-squared (RMS) voltage rectifier ($t_{½}$ = 3 s) to produce time-varying analog deflections on Triplet millivoltmeters (full-scale deflection equaled 100 μV) that reflected the RMS EMG activity.

Experimental Protocol

Twenty-two rats could not be entered into the study because their baseline EMG signals (using an a priori criteria of >4 μV RMS based on prior studies) contained excessive phasic activity (e.g., movement artifact) that precluded accurate measurement of basal muscle tone. The EMG signal from one rat was lost due to movement-induced electrode displacement after ATIP and before ALF injection; this rat was not included in the data analysis.

In the experimental groups, baseline electromyographic activity was measured for 15 min with EMG measurements at 5 and 10 min. At time 0, a final baseline measurement was obtained and then each animal received an SC injection of either saline (n = 18), ATIP 0.3 mg/kg (n = 19), or ATIP 1.0 mg/kg (n = 19). Electromyographic activity was recorded for an additional 10 min and then, just after the 10-min time point measurement, ALF was injected (50, 150, or 300 μg/kg, SC). Electromyographic activity was recorded for an additional 60 min following ALF injection. The timing of drug injections was based on preliminary studies and was designed to optimize the likelihood that the time of peak effect of the opiate agonist after systemic administration (5 min) coincided with the time during which the alpha$_2$ antagonist was maximally effective [(53) and unpublished].

The same protocol was used to study 12 control animals that were given saline instead of ALF to demonstrate the stability of the normally low levels of tonic EMG activity following placebo treatment (saline 1 ml/kg, SC; n = 6) and to examine the effects of pretreatment with atipamezole alone (1.0 mg/kg, SC; n = 6).

Data Analysis

Electromyographic data were recorded from each rat at 5-min intervals. Statistical differences between the two control groups were analyzed using two-way analysis of variance (ANOVA) with treatment (saline vs. ATIP) as the between-subjects factor and time as the within-subjects factor. Differences between ATIP/ALF treatment groups were then determined using a three-way ANOVA with correction for unequal group sizes using two between-subjects factors (ATIP dose and ALF dose) and one within-subjects factor (time) (55). These analyses used each animal as its own control such that the effects of ATIP and ALF were compared with baseline values. To determine specific drug-dose interactions, individual dose data were subsequently subjected to selected two-way ANOVA (groups and times). Newman-Keuls a posteriori tests [corrected for unequal group size using the harmonic mean (55)] were then performed to determine significant differences between groups. All data were expressed as mean ± SEM and a value of $p < 0.05$ was considered statistically significant.

RESULTS

One rat died after ATIP 1.0 mg/kg pretreatment and injection of 300 μg/kg of ALF. This animal seized prior to his demise and he was excluded from data analysis. Pretreatment with saline had no effect on baseline EMG activity (Fig. 1). Atipamezole (1 mg/kg) pretreatment, on the other hand, augmented EMG activity slightly [overall dose effect, $F(1, 10) = 11.7, p < 0.01$]. The difference between saline and ATIP was significant for up to 25 min following pretreatment injection, $F(1, 160) = 3.6$ to $10.6, p < 0.05$.

ALF significantly increased gastrocnemius muscle rigidity ($F = 5.3, p < 0.01$). However, this did not appear to be a dose-dependent effect (Fig. 2). The ATIP main effect did not attain statistical significance ($F = 2.8, p = 0.07$). At the highest ATIP dose, ALF 300 μg/kg produced significantly more rigidity than did ALF 50 or 150 μg/kg ($p < 0.05$). Ex-

amination of the dose-dose interaction revealed that the effect of ATIP was significant only at the highest ALF dose (300 μg/kg) group, $F(2, 47) = 5.2$, $p < 0.01$, whereas significant differentiation between ALF dose groups was only apparent after pretreatment with the highest ATIP dose, $F(2, 47) = 6.2$, $p < 0.005$.

All of the dose-time interactions were highly significant ($p < 0.001$). Across all ATIP pretreatment doses (ALF-time interaction), ALF produced significant muscle rigidity over time in all three ALF dose groups [$F(16, 752) = 2.7$, 4.9, and 18.7, respectively, all $p < 0.001$]. Compared with baseline values ($t = -10$, -5, and 0 on the figures), rigidity peaked transiently at 10 min post-ALF after the 50-μg/kg dose, but lasted for 25 min after the 150-μg/kg dose and for the entire 60 min following ALF 300 μg/kg. The EMG activity in the 50-μg/kg ALF dose was significantly lower than in the two higher dose groups at 15 min post-ALF. Rigidity after the 300-μg/kg ALF dose was significantly higher than in the 150 μg/kg dose beginning at 15 min post-ALF, and this difference continued for the duration of the experiment.

Across all ALF doses (ATIP-time interaction), ATIP significantly augmented muscle rigidity over time in both the 0.3- and 1.0-mg/kg pretreatment groups [$F(16, 752) = 8.3$ and 12.6, respectively, $p < 0.001$]. Although ATIP pretreatment prior to ALF failed to affect EMG activity significantly, following ALF there was a significant increase in EMG activity that persisted for 20 min in the ATIP 0.3-mg/kg dose group and for the entire 60-min post-ALF period in the ATIP 1.0-mg/kg dose group.

Effects of Atipamezole on Alfentanil-Induced Muscle Rigidity

Two-way ANOVAs were performed to examine in more detail the effects of ATIP on the ALF dose-time curves for muscle rigidity. Following saline pretreatment (Fig. 2), there was significant muscle rigidity in the 150- and 300-μg/kg ALF dose groups [$F(16, 256) = 4.7$ and 4.1, respectively, $p < 0.001$] but not in the 50-μg/kg dose group. The dose-time interaction between the three dose groups revealed several significant time points between 5 and 50 min post-ALF. The ALF 300-μg/kg dose group was significantly more rigid than the lowest ALF dose between 20 and 30 min post-ALF, whereas the ALF 150-μg/kg dose group was significantly higher than either of the other two groups at 60 min.

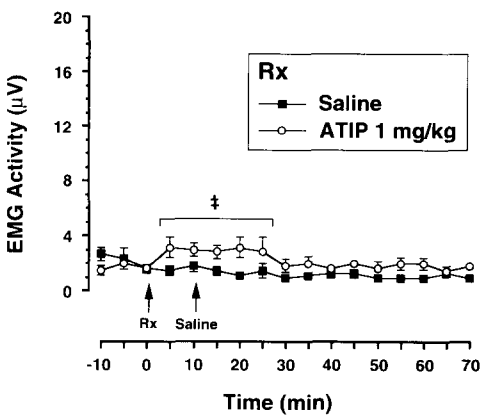

FIG. 1. The effects of saline or atipamezole (1.0 mg/kg) pretreatment on tonic gastrocnemius EMG activity (y-axis; μV RMS) are depicted over time (x-axis; minutes after SC injection). Saline was injected 10 min later (to mimic subsequent studies). EMG activity remained at low baseline levels for the duration of the study. Pretreatment with atipamezole produced a minor, but statistically significant, increase in basal EMG activity (‡$p < 0.05$) that persisted for up to 25 min postinjection.

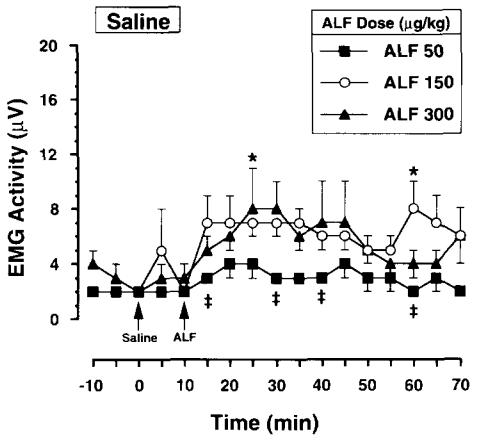

FIG. 2. The effects of alfentanil (50, 150, and 300 μg/kg) on tonic gastrocnemius EMG activity (y-axis; μV RMS) are depicted over time (x-axis; minutes after SC injection of saline). ALF (10 min after SAL injection) produced significant increases in EMG activity in both the 150- and 300-μg/kg groups when compared with baseline values by ANOVA, although the interaction was only significant at a few time points (*$p < 0.05$). The ALF 50-μg/kg dose produced significantly lower EMG values compared with either of the two higher doses (‡) at 5, 20, and 30 min post-ALF (15, 30, and 40 min post-ATIP, respectively). The 150-μg/kg value was significantly higher (‡) than both of the other two doses at the 60-min time point. Note that there is a greater than twofold increase in EMG activity after the two highest doses of ALF studied; this may be somewhat obscured by the use of a common y-axis EMG activity scale for all figures. Some error bars have been deleted to improve the clarity of the figure.

Following pretreatment with 0.3 mg/kg ATIP (Fig. 3), there was significant rigidity over time in all three dose groups [$F(16, 256) = 4.2$, 4.0, and 9.8, respectively, $p < 0.001$]. The rigidity in the 50- and 150-μg/kg dose groups was distinguishable from baseline between 5 and 10 min post-ALF. However, the 50- and 150-μg/kg doses were not significantly different from one another. In contrast, following ALF 300 μg/kg, muscle rigidity remained significantly higher than baseline values for the duration of the study. Between 20 and 55 min post-ALF, rigidity in the 300-μg/kg group was significantly greater than that in either of the two lower dose groups.

There was appreciable individual variability in EMG response in many of the animals pretreated with the highest dose of ATIP (1 mg/kg). Pretreatment with this ATIP dose (Fig. 4) resulted in significant rigidity only in the ALF 300-μg/kg dose group, $F(16, 256) = 10.1$, $p < 0.001$. In this group, EMG activity was significantly increased above baseline values for the duration of the study. The EMG activity in the ALF 300-μg/kg group was significantly greater than that in the 50-μg/kg dose group beginning 25 min post-ALF and, in the 150-μg/kg dose group, beginning 35 min post-ALF.

ATIP pretreatment augmented ALF-induced muscle rigidity in the 300-μg/kg ALF dose groups. There were significant increases in EMG activity over time after both ATIP 0.3 and 1.0 mg/kg pretreatment [$F(16, 272) = 2.8$ and 9.7, respectively, $p < 0.001$]. Dose-time interactions revealed significant rigidity in the 0.3-mg/kg ATIP dose group only at 5 min

post-ALF. However, in the ATIP 1.0-mg/kg group, significant rigidity persisted for the duration of the study. In fact, beginning 5 min after ALF injection, the EMG activity in the highest ATIP dose group was significantly greater than in the other two groups at nearly every time point.

DISCUSSION

The opiate agonist, alfentanil, increased the magnitude and duration of hindlimb muscle rigidity. Pretreatment with the alpha$_2$-adrenergic antagonist, atipamezole, increased muscle tone in control animals and also augmented muscle rigidity in animals given alfentanil. At the highest ATIP dose studied (1.0 mg/kg), both the intensity and the duration of opiate rigidity were increased. The ability of the alpha$_2$ antagonist both to enhance basal muscle tone and to augment opiate rigidity is consistent with the postulated neuropharmacological effects of alpha-adrenergic and opioid systems in the mediation of muscle tone and the control of posture.

Recent studies using animal models of opiate rigidity have shown that alpha$_2$-adrenergic agonists prevent opiate-induced rigidity (23,46,51). Brain sites thought to play a role in opiate rigidity (28,49,52) contain a significant number of adrenergic receptors (47). Dexmedetomidine pretreatment prevents ALF rigidity in the spontaneously ventilating rat via a central site of action (51). Yohimbine, a much less potent or selective alpha$_2$ antagonist than ATIP (26), appeared to potentiate fentanyl-induced muscle rigidity in the ketamine-anesthetized, mechanically ventilated rat (46). Lui et al. found that an alpha$_1$ antagonist, prazosin, but not yohimbine, blocked the rigidity induced by direct injections of fentanyl into the locus coeruleus (29).

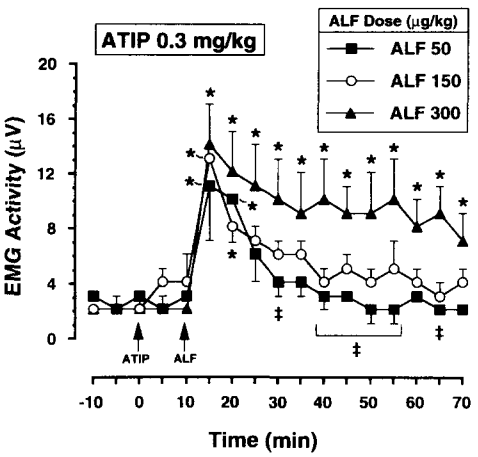

FIG. 3. Pretreatment with ATIP (0.3 mg/kg) at time zero resulted in an amplification of the dose-dependent effects of alfentanil (50–300 μg/kg) on tonic gastrocnemius EMG activity (y-axis; μV RMS). After ATIP 0.3 mg/kg, significant muscle rigidity could be demonstrated in all three ALF dose groups. Although the EMG activity in the ALF 50- and 150-μg/kg groups was significantly greater than baseline values for 10 min post-ALF (*$p < 0.05$), a sustained increase (*$p < 0.05$ compared with baseline for the entire study duration) in muscle activity could be demonstrated after ALF 300 μg/kg. The highest ALF dose resulted in significantly greater rigidity compared with the two lower doses at several time points between 20 and 55 min post-ALF (‡$p < 0.05$).

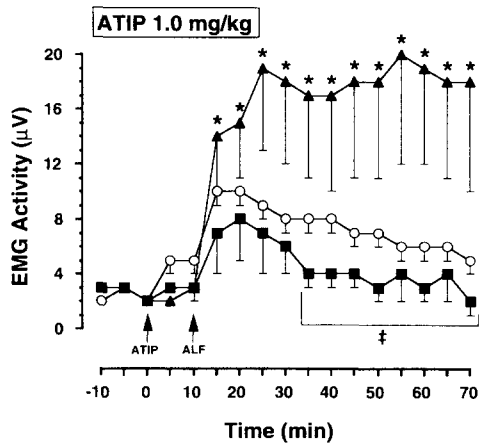

FIG. 4. Pretreatment with ATIP (1.0 mg/kg) produced a significant increase in alfentanil-induced muscle rigidity in the 300-μg/kg (*$p < 0.05$ compared with baseline for the entire post-ALF period) but not in the 50 (■)- or 150-μg/kg (○) ALF dose groups. The increased EMG activity in the ALF 300-μg/kg (▲) group attained statistical significance when compared with the 50- and 150-μg/kg dose groups (‡$p < 0.05$) beginning 25 min post-ALF. ATIP pretreatment appeared to result in an increased variability of response to ALF, and this effect diminished the ability to obtain statistically significant differences between the three ALF doses studied.

In a preliminary study, ATIP not only ameliorated the effects of DEX on ALF rigidity, but administration of the antagonist was accompanied by an apparent increase in ALF-induced muscle rigidity (unpublished data). These findings, in combination with the research discussed above, led to the hypothesis that alpha$_2$ antagonists would, in and of themselves, augment opiate-induced muscle rigidity. This prediction is supported by the results of the present study.

The alpha$_2$ agonist, DEX, produces muscle flaccidity and a decrease in peripheral EMG activity (49). If alpha-adrenergic systems play a role in normal muscle tone, then it might be postulated that ATIP, when administered alone, would increase EMG activity. This, in fact, was demonstrated in the control experiment. In previous work, ATIP, in doses up to 3.0 mg/kg, produced few gross behavioral effects (39). However, this earlier study did not examine muscle tone or postural support mechanisms. More recently, rodent studies documented suppression of locomotor activity in open field tests (26) and, at higher doses, increased anxiety, rearing behaviors, and apparent hostility (39). Nevertheless, the results of the present study in which ATIP augmented basal EMG activity are consistent with other research (15) suggesting that tonically active central alpha-adrenergic systems play a role in the control of muscle tone.

The ability of the highly selective alpha$_2$ antagonist ATIP to augment ALF-induced muscle rigidity implies that noradrenergic systems may influence opiate rigidity in one of two ways: either endogenous adrenergic neuronal activity plays a direct modulating role in the expression of opiate rigidity or alpha$_2$ adrenoceptors located on opioidergic neurons, under tonic adrenergic activity, are integral to the expression of opiate-induced muscular hypertonus.

Opioid receptors and alpha$_2$ adrenoceptors are colocalized

in many areas of the mammalian brain (35). These two receptor classes share similar signal transduction mechanisms; both utilize pertussis toxin-sensitive G-proteins (1) and are linked to potassium channels (21,33). Thus, the site of interaction of these two ligand classes could be either in the neuronal membrane between the colocalized receptors themselves or in their common signal transduction systems (21).

Alpha$_2$ agonists augment opiate analgesia both centrally (31,47) and in the spinal cord (34,56). Naloxone antagonizes some forms of alpha$_2$ analgesia (30), and there is cross-tolerance between some types of alpha$_2$ and opiate antinociception (4). It has been suggested that alpha$_2$ agonists produce their analgesic effects via activation of central opioid systems (31,56). Alpha$_2$ agonists also appear to augment opiate anesthesia (18). Alpha$_2$-adrenergic and opioid receptors are both found in brain regions that have been documented to play a role in analgesia (47) and perhaps anesthesia (13).

It is now widely believed, based on data from in vitro and in vivo pharmacology, that there are at least three (A, B, and C) alpha$_2$ subtypes or isoreceptors (10). At least three different alpha$_2$ adrenoceptor gene products (C2, C4, and C10) have been isolated (38). The functional significance of these alpha$_2$ isoreceptors is still being clarified. In vivo pharmacology has thus far been able to distinguish alpha$_2$ agonists on the basis of A vs. non-A isoreceptor-mediated effects. For example, studies of the spinal antinociceptive effects of alpha$_2$ agonists and antagonists suggest that atipamezole, like dexmedetomidine, acts predominantly at the alpha$_{2A}$ isoreceptor (44,45).

The results of the present study, as well as the finding that intracerebroventricular injection of dexmedetomidine blocks alfentanil rigidity (27), support the hypothesis that the alpha$_{2A}$ receptor plays a role in opiate rigidity. However, in preliminary studies, the alpha$_2$ non-A-preferring agonist ST91 (45), when administered ICV, also antagonized opiate rigidity (27). The slopes of the agonist dose–effect curves for DEX and ST91 were nearly parallel. Thus, the precise role of these two isoreceptors in opiate rigidity remains to be clarified.

It is widely believed that alpha$_2$ agonists produce their supraspinal sedative and antinociceptive effects via binding to prejunctional adrenergic receptors resulting in decreased release of norepinephrine (11,31). However, more recent data suggest that the central anesthetic effects of dexmedetomidine are mediated by both pre- and postsynaptic receptors (42), possibly in the locus coeruleus (12). It is not possible, based on the results of the present study, to ascertain whether the augmentation by atipamezole of opiate-induced rigidity is due to a pre- or postsynaptic site of action.

The intriguing possibility that the effects of atipamezole on opiate-induced rigidity reflects an interaction between alpha$_2$ and 5-HT systems deserves comment. In a previous study in our laboratory, ketanserin, a type 2 serotonergic antagonist (22), was shown to attenuate alfentanil-induced muscle rigidity (50). A potential role for serotonergic systems in opiate rigidity is also supported by the finding that intraventricular 5,7-dihydroxytryptamine prevents the rigidity associated with injections of beta-endorphin into the periaqueductal gray (54).

The nucleus raphe pontis, a site known to play a role in opiate-induced muscle rigidity (8,49,52), is a source of ascending, descending, and cerebellar serotonergic efferents (7,17, 43) as well as containing adrenergic, opioid, cholinergic, and peptidergic receptors (9). Several studies have documented the close physiological and anatomical relationships between adrenergic, serotonergic, and opioid neural pathways, particularly in brain stem areas known to mediate the behavioral manifestations of opiate anesthesia (5,14). Alpha$_2$ adrenoceptors have been postulated to modulate serotonin biosynthesis (58) as well as the firing of 5-HT neurons in the dorsal raphe (3). It has also been suggested that serotonergic systems regulate central alpha$_2$ adrenoceptors (37). In the case of opiate rigidity, the relative roles of alpha$_2$-adrenergic and serotonergic systems remain to be clarified.

Possible Limitations of the Present Study

Because atipamezole was administered subcutaneously, it is possible that the effects observed could have been due to a peripheral site or mechanism of action. However, several factors argue in favor of the observed effects being due to a central action. Atipamezole readily enters the brain after systemic administration (48). The ability of alpha$_2$ agonists to attenuate opiate rigidity is a central effect (27,51). Finally, in a pilot study, intracerebroventricular injection of atipamezole (40 μg/10 μl) completely reversed the ameliorating effects of pretreatment with dexmedetomidine (30 μg/kg, SC, 15 min earlier) on the muscle rigidity produced by alfentanil (500 μg/kg, SC) (unpublished data). Additional studies using either systemic injections of a selective alpha$_2$ antagonist like DG5128, which does not cross the blood–brain barrier (51), or central injections of atipamezole would help to exclude a peripheral site of action.

Because tissue or plasma drug levels were not measured, it is not possible to determine whether the effects of atipamezole on opiate rigidity are due to pharmacokinetic or pharmacodynamic factors. For example, it is conceivable that ATIP pretreatment, as a result of cardiovascular or respiratory depression, could have altered the uptake or distribution of ALF. However, ATIP has significantly less cardiorespiratory effects than the alpha$_2$ agonist DEX (39,41) and, in a previous study (16), we demonstrated that DEX did not augment the cardiovascular or respiratory depression produced by high-dose alfentanil. In any case, the present data have clinical relevance: the administration of an alpha$_2$ antagonist to an animal or human anesthetized with large doses of opiates will, in the absence of muscle paralysis, likely result in increased muscle tone.

In the course of studying opiate-induced muscle rigidity in over 3000 rats over the last 7 years, it became readily apparent that there was tremendous individual variability in this phenomenon. In another recent study, using Wistar rats from the same vendor (Harlan), alfentanil 300 μg/kg produced peak EMG activity levels of over 20 μV RMS (57), whereas in the present study, saline-pretreated rats given the same dose of ALF only attained peak values of about 8 μV (a value that is much lower than those observed in most previous studies in our laboratory). In addition to possible genetic factors related to the batch of rats studied, environmental factors that may also affect the variability of rigidity observed in different studies include time of day, season of the year, ambient noise in and around the laboratory, and the handling skill of the technician performing the studies. For these reasons, the present study was designed (prior to initiating any experiment) to include random allocation to treatment groups and blinded experimental observers. Nevertheless, the variability of response of the animals included in the present study was greater than we have seen in any previous work. This increased variability could also be due, in part, to some unappreciated effect of the alpha$_2$ antagonist under investigation. Nevertheless, sig-

nificant effects of atipamezole treatment on both basal muscle tone and on ALF rigidity were still observed.

Conclusions

In contrast to alpha$_2$ antagonist reversal of opiate antinociception (20), the present study shows that alpha$_2$ antagonists augment basal muscle tone and also opiate-induced muscle rigidity. In an earlier study, the alpha$_2$ agonist dexmedetomidine antagonized ALF rigidity (51). These findings appear to support the hypothesis that opiate-induced muscle rigidity is mediated, at least in part, by an endogenous alpha$_2$-adrenergic system. It also appears that a different neuroanatomical site and/or mechanism of action is responsible for the alpha$_2$-adrenergic mediation of opiate rigidity compared with opiate analgesia and anesthesia.

ACKNOWLEDGEMENTS

The editorial assistance of Joan Azar and the technical assistance of Cory Campbell, David Wood, and Philip Cole were greatly appreciated. Preliminary work, performed by Deborah Taurek, was important to the planning of this study. Dr. George Koob provided valuable editorial assistance. This research was supported in part by National Institute on Drug Abuse grant DA06616A.

REFERENCES

1. Aghajanian, G. K.; Wang, Y.-Y. Pertusis toxin blocks the outward currents evoked by opiate and alpha-2 agonists in locus coeruleus neurons. Brain Res. 371:390–394; 1986.
2. Aho, M.; Erkola, O.; Kallio, A.; Scheinin, H.; Kortilla, K. Comparison of alpha-2 agonist dexmedetomidine and midazolam and antagonism of dexmedetomidine with atipamezole. Anesth. Analg. 74:S3; 1992.
3. Baraban, J. M.; Aghajanian, G. K. Suppression on firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenergic antagonists. Neuropharmacology 19:355–363; 1980.
4. Bartoletti, M.; Gairdi, M.; Gubellini, C.; Bacchi, A.; Babbini, M. Cross tolerance between morphine and clonidine: A study on motility in rats. Neuropharmacology 28:1159–1162; 1989.
5. Basbaum, A.; Fields, H. Endogenous pain control systems: Brainstem spinal pathways and endorphin circuitry. Annu. Rev. Neurosci. 7:309–338; 1984.
6. Belleville, J. P.; Ward, D. S.; Bloor, B. C.; Maze, M. Ventilatory effects of dexmedetomidine in humans. Anesthesiology 73: A1167; 1990.
7. Bjorklund, A.; Nobin, A.; Stenevi, V. The use of neurotoxic dihydroxytryptamines as a tool for morphological studies and localized lesioning of central indoleamine neurons. Z. Zellforsch. 145:479–501; 1979.
8. Blasco, T. A.; Lee, D. E.; Amalric, M.; Swerdlow, N. R.; Smith, N. T.; Koob, G. F. The role of the nucleus raphe pontis and the caudate nucleus in alfentanil rigidity in the rat. Brain Res. 386: 280–286; 1986.
9. Bowker, R.; Westlund, K.; Sullivan, M.; Wilber, J.; Coulter, J. Descending serotonergic, peptidergic, and cholinergic pathways from the raphe nuclei: A multiple transmitter complex. Brain Res. 288:33–48; 1983.
10. Bylund, D. B. Subtypes of alpha-2 adrenoceptors: Pharmacological and molecular biological evidence converge. Trends Pharmacol. 9:356–361; 1988.
11. Chance, W. T. Clonidine analgesia: Tolerance and cross-tolerance. Life Sci. 33:2241–2246; 1983.
12. Correa-Sales, C.; Rabin, B. C.; Maze, M. A hypnotic response to dexmedetomidine, an alpha-2 agonist, is mediated in the locus coeruleus in rats. Anesthesiology 76:948–952; 1992.
13. Correa-Sales, C.; Rabin, R. C.; Reid, K.; Maze, M. Ribosylation of G proteins in the locus coeruleus of rats prevents the hypnotic/anesthetic action of the alpha-2 adrenergic agonist, dexmedetomidine. Anesth. Analg. 74:S57; 1992.
14. Frankhuijzen, A. L.; Wardeh, G.; Hogenboom, F.; Mulder, A. H. Alpha-2-adrenoceptor mediated inhibition of the release of radio-labelled 5-hydroxytryptamine and noradrenaline from slices of the dorsal raphe region of the rat brain. Naunyn Schmiedebergs Arch. Pharmacol. 337:255–260; 1988.
15. Fung, S. J.; Manzoni, D.; Chan, J. Y. H.; Pompeiano, O.; Barnes, C. D. Locus coeruleus control of spinal motor output. In: Barnes, C. D.; Pompeiano, O., eds. Progress in brain research. Amsterdam: Elsevier Science Publishers; 1991:395–409.
16. Furst, S. R.; Weinger, M. B. Dexmedetomidine, a selective alpha-2 agonist, does not potentiate the cardiorespiratory depression of alfentanil in the rat. Anesthesiology 72:882–888; 1990.
17. Geyer, M. A.; Puerto, A.; Dawsey, W. J.; Knapp, S.; Bullard, W. P.; Mandell, A. J. Histologic and enzymatic studies of the mesolimbic and mesostriatal serotonergic pathways. Brain Res. 106:241–256; 1976.
18. Horvath, G.; Szikszay, M.; Rubicsek, G.; Benedek, G. An isobolographic analysis of the hypnotic effects of combinations of dexmedetomidine with fentanyl or diazepam in the rat. Life Sci. 50: PL216–PL220; 1992.
19. Hu, C.; Flecknell, P.; Liles, J. Fentanyl and medetomidine anaesthesia in the rat and its reversal using atipamezole and either nalbuphine or butorphanol. Lab. Anim. 26:15–22; 1992.
20. Iglesias, V.; Alguacil, L. F.; Alamo, C.; Cuenca, E. Effects of yohimbine on morphine analgesia and physical dependence in the rat. Eur. J. Pharmacol. 211:35–38; 1992.
21. Illes, P.; Norenberg, W. Blockade of alpha-2 adrenoceptors increases opioid mu-receptor-medicated inhibition of the firing rate of rat locus coeruleus neurones. Naunyn Schmiedebergs Arch. Pharmacol. 342:490–496; 1990.
22. Janssen, P. A. J. Pharmacology of potent and selective S2-serotonergic antagonists. J. Cardiovasc. Pharmacol. 7(Suppl. 7): S2–S11; 1985.
23. Jerussi, T. P.; Capacchione, J. F.; Benvenga, M. J. Reversal of opioid-induced muscular rigidity in rats: Evidence for alpha-2 adrenergic involvement. Pharmacol. Biochem. Behav. 28:283–289; 1987.
24. Karhuvaara, S.; Kallio, A.; Salonen, M.; Tuominen, J.; Scheinin, M. Rapid reversal of alpha-2 adrenoceptor agonist effects by atipamezole in human volunteers. Br. J. Clin. Pharmacol. 31:160–165; 1991.
25. Karhuvaara, S.; Kallio, A.; Scheinin, M.; Anttila, M.; Salonen, J.; Scheinin, H. Pharmacological effects and pharmacokinetics of atipamezole, a novel alpha-2 adrenoceptor antagonist—a randomized, double-blind cross-over study in healthy male volunteers. Br. J. Clin. Pharmacol. 30:97–106; 1990.
26. Kauppila, T.; Tanila, H.; Carlson, S.; Taira, T. Effects of atipamezole, an alpha-2 adrenoceptor antagonist, in open-field, plus-maze, two compartment exploratory, and forced swimming tests in the rat. Eur. J. Pharmacol. 205:177–182; 1991.
27. Lin, T.; Lau, C.; Weinger, M. B. Elucidation of the neuropharmacology of opiate-induced muscle rigidity in the rat. Anesthesiology 77:A734; 1992.
28. Lui, P. W.; Lee, T. Y.; Chan, S. H. H. Involvement of locus coeruleus and noradrenergic neurotransmission in fentanyl-induced muscular rigidity in the rat. Neurosci. Lett. 96:114–119; 1989.
29. Lui, P. W.; Lee, T. Y.; Chan, S. H. H. Involvement of coerulospinal noradrenergic pathway in fentanyl-induced muscular rigidity in rats. Neurosci. Lett. 108:183–188; 1990.
30. Mastrianni, J. A.; Abbott, F.; Kunos, G. The activation of central mu-opioid receptors is involved in clonidine analgesia in rats. Brain Res. 479:283–289; 1989.
31. Maze, M.; Tranquilli, W. Alpha-2 adrenoceptor agonists. Anesthesiology 74:581–605; 1991.
32. Nguyen, D.; Abdul-Rasool, I.; Ward, D.; Hsieh, J.; Kobayashi, D.; Hadlock, S.; Singer, F.; Bloor, B. Ventilatory effects of dex-

medetomidine, atipamezole, and isoflurane in dogs. Anesthesiology 76:573–579; 1992.
33. North, R. A.; Williams, J. T.; Surprenant, A.; Christie, M. J. Mu and delta receptors belong to a family of receptors that are coupled to potassium channels. Proc. Natl. Acad. Sci. USA 84: 5487–5491; 1987.
34. Ossipov, M. H.; Harris, S.; Lloyd, P.; Mèssineo, E. An isobolographic analysis of the antinociceptive effect of systemically and intrathecally administered combinations of clonidine and opiates. J. Pharmacol. Exp. Ther. 255:1107–1116; 1990.
35. Pazos, A. Anatomical distribution of opioid receptors and adrenoceptors in the brain of mammals. Rev. Farmacol. Clin. Exp. 5(Suppl. 1):11–17; 1988.
36. Pertovaara, A.; Kauppila, T.; Tukeva, T. The effect of medetomidine, an alpha-2 adrenoceptor agonist, in various pain tests. Eur. J. Pharmacol. 179:323–328; 1990.
37. Rappaport, A.; Sturtz, F.; Guicheney, P. Regulation of central alpha-adrenoceptors by serotoninergic denervation. Brain Res. 344:158–161; 1985.
38. Ruffollo, R. R.; Nichols, A. J.; Stadel, J. M.; Hiebel, J. P. Pharmacologic and therapeutic applications of alpha-2 adrenoceptor subtypes. Annu. Rev. Phamacol. Toxicol. 32:243–279; 1993.
39. Scheinin, H.; MacDonald, E.; Scheinin, M. Behavioral and neurochemical effects of atipamezole, a novel alpha-2 adrenoceptor antagonist. Eur. J. Pharmacol. 151:35–42; 1988.
40. Scheinin, H.; Virtanen, R.; Macdonald, E.; Lammintausta, R.; Scheinin, M. Medetomidine—a novel alpha-2-adrenoceptor agonist: A review of its pharmacodynamic effects. Prog. Neuropsychopharmacol. 13:635–651; 1989.
41. Scheinin, M.; Kallio, A.; Koulu, M.; Viikari, J.; Schenin, H. Sedative and cardiovascular effects of medetomidine, a novel selective alpha-2 adrenoceptor agonist, in healthy volunteers. Br. J. Clin. Pharmacol. 24:443–451; 1987.
42. Segal, I. S.; Dose, V.; Sheridan, B. C.; Vickery, R. G.; Maze, M. Does medetomidine decrease anesthetic requirements through both pre- and postsynaptic alpha-2 adrenoreceptors? Anesthesiology 69:818–823; 1988.
43. Steinbusch, H. W. M. Serotonin-immunoreactive neurons and their projections in the CNS. In: Bjorklund, A.; Hokfelt, T.; Kuhar, M. J., eds. Handbook of chemical neuroanatomy. New York: Elsevier Science Publishers; 1984:68–96.
44. Takano, Y.; Takano, M.; Yaksh, T. L. The effect of intrathecally administered imiloxan and WB4101: Possible role of alpha-2 adrenoceptor subtypes in the spinal cord. Eur. J. Pharmacol. 219: 465–468; 1992.
45. Takano, Y.; Yaksh, T. L. Characterization of the pharmacology of intrathecally administered alpha-2 agonists and antagonists in rats. J. Pharmacol. Exp. Ther. 261:764–772; 1992.
46. Tsou, M. Y.; Lui, P. W.; Lee, T. Y.; Pan, J. T.; Chan, S. H. H. Differential effects of prazosin and yohimbine on fentayl-induced muscular rigidity in rats. Neuropharmacology 28:1163–1168; 1989.
47. Unnerstall, J. R.; Kopajtic, T. A.; Kuhar, M. J. Distribution of alpha-2 agonist binding sites in the rat and human central nervous system: Analysis of some functional, anatomic correlates of the pharmacological effects of clonidine and related adrenergic agents. Brain Res. Rev. 7:69–101; 1984.
48. Virtanen, R.; Savola, J.; Saano, V. Highly selective and specific antagonism of central and peripheral alpha-2 adrenoceptors by atipamezole. Arch. Int. Pharmacodyn. 297:190–204; 1989.
49. Weinger, M. B.; Cline, E. J.; Smith, N. T.; Blasco, T. A.; Koob, G. F. Localization of brainstem sites which mediate alfentanil-induced muscle rigidity in the rat. Pharmacol. Biochem. Behav. 29:573–580; 1988.
50. Weinger, M. B.; Cline, E. J.; Smith, N. T.; Koob, G. F. Ketanserin pretreatment reverses alfentanil-induced muscle rigidity. Anesthesiology 67:348–354; 1987.
51. Weinger, M. B.; Segal, I. S.; Maze, M. Dexmedetomidine, acting through central alpha-2 adrenoceptors, prevents opiate-induced muscle rigidity in the rat. Anesthesiology 71:242–249; 1989.
52. Weinger, M. B.; Smith, N. T.; Blasco, T. A.; Koob, G. F. Brain sites mediating opiate-induced muscle rigidity in the rat: Methylnaloxonium mapping study. Brain Res. 544:181–190; 1991.
53. Weinger, M. B.; Taurek, D. L. The effects on muscle tone of selective alpha-2 adrenergic agonists and antagonists during high-dose alfentanil anesthesia in the rat. Anesth. Analg. 70:S425; 1990.
54. Widdowson, P. S.; Griffiths, E. C.; Slater, P. The effects of opioids in the periaqueductal gray region of rat brain on hindlimb muscle tone. Neuropeptides 7:251–258; 1986.
55. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1971.
56. Yaksh, T. L. Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. Pharmacol. Biochem. Behav. 22:845–858; 1985.
57. Yang, P.; Weinger, M. B.; Negus, S. S. Dose-effect studies of different end-points of opiate anesthesia in the rat. Anesthesiology 77:153–161; 1992.
58. Yoshioka, M.; Matsumoto, M.; Togashi, H.; Smith, C. B.; Saito, H. Alpha-2 adrenoceptor modulation of biosynthesis in the rat brain. Neurosci. Lett. 139:53–56; 1992.