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Clonidine Suppresses Methylxanthine Induced Quasi-Morphine Withdrawal Syndrome

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GRANT, S. J. AND D. E. REDMOND, JR. *Clonidine suppresses methylxanthine induced quasi-morphine withdrawal syndrome*. PHARMAC. BIOCHEM. BEHAV. 17(4) 655–658, 1982.—The effects of the alpha-2 adrenergic agonist, clonidine, on the "quasi-morphine withdrawal syndrome" (QMWS) were examined in drug naive rats. The QMWS was induced by combined systemic administration of iso-butyl-methylxanthine (IBMX: 15 mg/kg, IP) and naloxone (1 mg/kg, IP). Pretreatment with clonidine (50 μg/kg, IP) significantly decreased the incidence of 11 out of 16 withdrawal signs. Since clonidine suppresses signs and symptoms of true morphine withdrawal, the suppression of methylxanthine effects demonstrates an additional similarity of the QMWS to true morphine withdrawal. These results suggest that a significant common neural mechanism of both the QMWS and true morphine withdrawal is affected by clonidine.

Morphine withdrawal Quasi-morphine withdrawal syndrome Methylxanthines Iso-butyl-methylxanthine Noradrenaline Clonidine

METHYLXANTHINES evoke behaviors associated with opiate withdrawal in both opiate dependent and drug naive animals [7,8]. The synthetic methylxanthine iso-butyl methylxanthine (IBMX) is the most potent one in this regard, but others, including the widely consumed methylxanthines caffeine and theophylline, as well as some non-xanthine compounds, produce similar behavioral effects [9]. Because of specific behavioral, pharmacological, and neurochemical parallels with morphine withdrawal, this phenomenon has been termed the quasi-morphine withdrawal syndrome (QMWS), and it has been suggested that both withdrawal syndromes share common neural substrates [7, 8, 9].

One criterion suggested for the characterization of the QMWS is that non-opiate drugs should have effects on the QMWS comparable to their effects on morphine withdrawal [8]. Relatively low doses of clonidine have been shown to reduce morphine withdrawal signs and symptoms in humans [6,14] as well as the behavioral effects of morphine withdrawal in a number of animal experimental paradigms [12, 17, 23]. The present study was undertaken to determine whether clonidine suppressed the QMWS induced by IBMX. If so, the present data combined with the results of previous studies would provide further support for a fundamental similarity between the methylxanthine-induced QMWS and true morphine withdrawal.

METHOD

Male albino rats (250–400 g) from commercial sources (Charles River) were treated with one or more of the following: iso-butyl-methyl-xanthine (Sigma), clonidine HCl (Boehringer Ingelheim), Naloxone HCl (Endo Lab.). Both clonidine and naloxone were dissolved in normal saline. IBMX was dissolved in non-aqueous diazepam vehicle (40% propylene glycol, 10% ethyl alcohol, 1.5% sodium benzoate, 5% benzl alcohol, Hoffman-LaRoche).

The effect of clonidine on the behaviors of the QMWS was examined in 24 subjects. Drug administration began following 30 min habituation to the 24×46×42 cm enclosed Plexiglas testing chamber. Following pretreatment with either clonidine (50 μg/kg, IP; N=12) or an equal volume of saline (N=12), the QMWS was induced 15 min later by IP administration of 15 mg/kg IBMX and 1 mg/kg naloxone. The combination of IBMX and naloxone has been reported to be the most effective in eliciting the QMWS [8]. The dose of 50 μg/kg (IP) has previously been reported to be effective in suppressing the behavioral effects of opiate withdrawal in rodents [12,23], and reversing the effect of opiate withdrawal in rodents [10]. Behavioral observation began 5 min later and continued during the next 15 minutes. Behaviors previously associated with both true morphine withdrawal [3] and the

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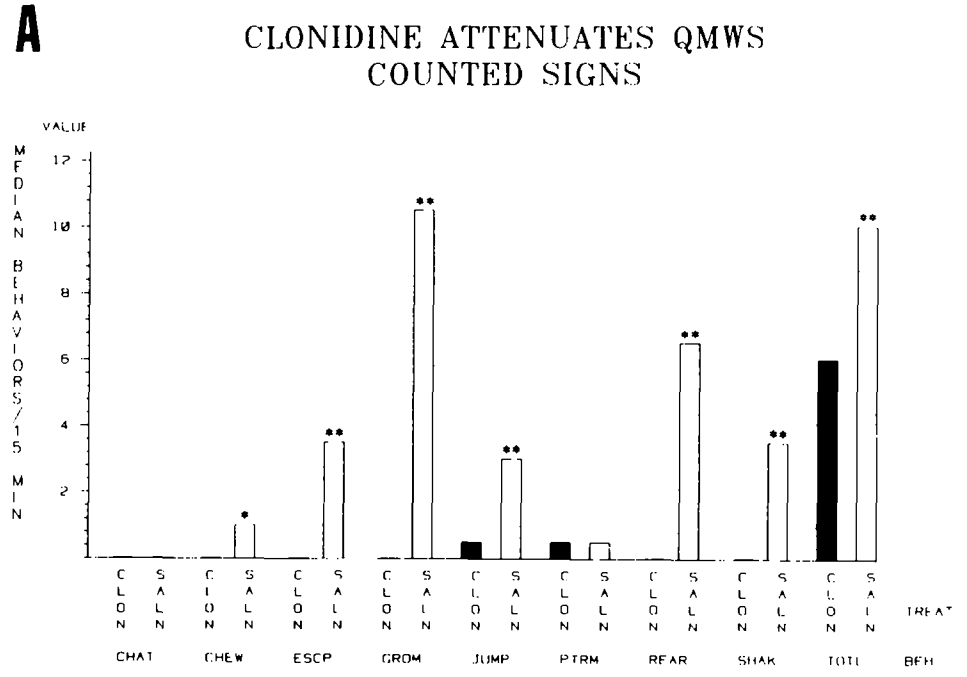

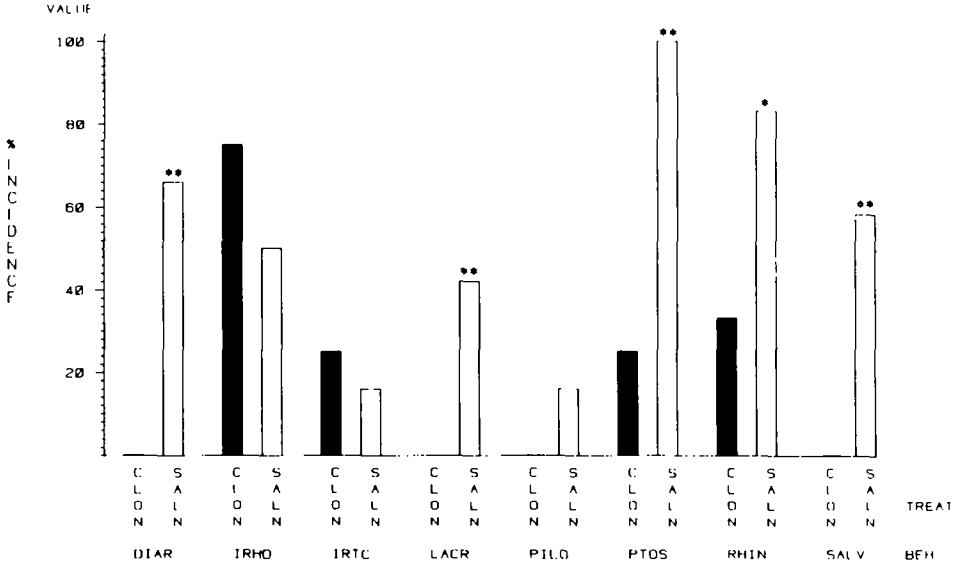

FIG. 1. Effect of clonidine on the occurrence of Quasi-Morphine Withdrawal Syndrome (QMWS) induced by combined administration of IBMX (15 mg/kg IP) and naloxone (1.0 mg/kg IP). Clonidine (CLON: 50 μg/kg IP, N = 12) or saline (SAL: N = 12) was administered 15 min prior to IBMX/Naloxone injections. (A) Counted signs: results expressed as median number of occurrences of sign during 15 min observation period, $^{*}p<0.05$, $^{**}p<0.01$ Mann-Whitney U test. (B) Checked signs: results expressed as percent of subjects exhibiting sign during 15 min observation period, $^{*}p<0.05$, $^{**}p<0.01$ Fisher's exact probability test.

QMWS [7,26] were rated by observers blind to the nature of the pretreatment (Table 1). Counted signs were analyzed with a Mann-Whitney U test; checked signs by Fisher's exact probability test.

RESULTS

Subjects pretreated with saline and then treated with IBMX and naloxone exhibited behaviors of similar type and intensity to the QMWS reported by other investigators (Fig. 1) [7,26]. However, clonidine pretreatment significantly attenuated the incidence of 11 of 16 signs of the QMWS (Fig. 1). Five behaviors were not significantly affected by clonidine, but 2 of these (teeth chattering and paw tremor) occurred infrequently even in the subjects treated with saline prior to IBMX and naloxone.

DISCUSSION

The suppression by clonidine of some of the behaviors induced by IBMX illustrates a further similarity between the QMWS and true morphine withdrawal. These results correspond to clinical [6,14] and behavioral [12, 17, 23] studies in which clonidine suppressed various effects of morphine withdrawal. Thus, clonidine's effects on the QMWS are comparable to its effect on morphine withdrawal. This similarity is consistent with the hypothesis that the QMWS and true morphine withdrawal involve common neural processes [8].

Methylxanthines have a broad pharmacological spectrum of actions which affect a number of basic cellular processes, including inhibition of phosphodiesterase, antagonism of adenosine and interference with calcium currents. These broad actions make it difficult to determine the exact molecular mechanism underlying the QMWS [11,22]. Methylxanthines increase single unit activity of noradrenergic neurons in the LC [15], and increase the brain concentration of the noradrenergic metabolite, MHPG, [13] and clonidine reverses such increases. Hyperactivity of central noradrenergic neurons is also seen during true morphine withdrawal, and this hyperactivity is reduced by clonidine [1,10]. A critical role for noradrenaline during morphine withdrawal is consistent with the prominent endogenous opiate projections to central noradrenergic neurons [4], the high opiate receptor density on noradrenergic neurons [19], and the potent inhibition by endogenous and exogenous opiates of noradrenergic activity [2, 16, 18, 25]. In addition, activation of the locus coeruleus by electrical stimulation produces some opiate withdrawal signs in non-human primates [20], and this effect can be blocked by clonidine [20,21]. These studies suggest that hyperactivity of central noradrenergic neurons may be a critical common aspect of both true morphine withdrawal and the QMWS. The fact that clonidine acts primarily, but not exclusively, as an agonist at central alpha-2 adrenergic receptors to decrease both release of noradrenaline from terminals and the firing of noradrenergic neurons [5,24], suggests that activation of central NA neurons may be a neural substrate of the QMWS. However, since both methylxanthines and opiates have effects on cells besides those of the central noradrenergic system, the participation of other neural and even extra-neural systems in true morphine withdrawal syndrome and the QMWS can not be ignored.

It is unlikely that the observed effects of clonidine are due to a peripheral site of action since a clonidine analogue which does not enter the central nervous system does not affect true morphine withdrawal in rats and dogs [17]. It would be informative now to determine whether the anti-withdrawal actions of clonidine generalize to other xanthines and non-xanthine agents which elicit the QMWS [9].

TABLE 1

QUASI-MORPHINE WITHDRAWAL SYNDROME BEHAVIORAL SIGNS

Behaviors Checked as Present or Absent During Observation Period	
Diarrhea (DIAR)	Loose or watery stools.
Irritable to Handling (IRHD)	Vocalization when lightly grasped.
Irritable to Touch (IRTC)	Vocalization when lightly stroked with object.
Lacrimination (LACR)	Noticeable secretion from eyes.
Piloerection (PILO)	Hair standing on end, especially along dorsal surface.
Rhinorrhea (RHINO)	Noticeable secretion from nose.
Salivation (SALV)	Noticeable secretion from mouth.
Behaviors Counted Per Occurrence During Observation Period	
Teeth Chattering (CHAT)	Audible grinding of teeth.
Chewing (CHEW)	Undirected jaw movements.
Escape (ESCP)	Digging or treading motion with forelimbs or rubbing jaw along floor.
Grooming (GROM)	Licking or biting directed towards body or limbs.
Jumping (JUMP)	Leaping with all four legs off floor.
Paw Tremor (PTRM)	Shaking or flicking of forelimbs.
Rearing (REAR)	Standing on hindlimbs with forelimbs off floor.
Wet Dog Shakes (SHAK)	Rapid tremor or shaking of head, upper torso or whole body.
Total (TOTL)	Total number of behavioral signs exhibited by a subject

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