Pharmacology Biochemistry & Behavior, Vol. 28, pp. 235–241. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Attenuation of Isobutylmethylxanthine-Induced Suppression of Operant Behavior by Pretreatment of Rats With Clonidine[3]

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Received 12 February 1987

KLEVEN, M. S. AND S. B. SPARBER. *Attenuation of isobutylmethylxanthine-induced suppression of operant behavior by pretreatment of rats with clonidine.* PHARMACOL BIOCHEM BEHAV **28**(2) 235–241, 1987.— Administration of 3-isobutyl-1-methylxanthine (IBMX) to rats performing a FR30 operant for food reinforcement produces a dose-dependent suppression of behavior. Operant behavior suppressed by 5 mg IBMX/kg is attenuated by pretreatment, 30 min before the operant session, with the α_2 adrenergic agonist clonidine (5–30 μg/kg). Clonidine itself causes a dose-dependent reduction in FR30 responding prior to the administration of IBMX. However, doses of clonidine which also suppressed responding were not more effective than lower doses in attenuating the suppression of operant behavior caused by IBMX, perhaps due to postsynaptic or nonspecific actions of clonidine. Methylxanthines, alone or in combination with the opiate antagonist naloxone, produce signs of opiate withdrawal. This quasi-morphine withdrawal syndrome may be useful in studies of either the development or expression of opiate withdrawal. Since clonidine attenuates the rate-suppressant effect of IBMX, it is likely that a significant component of IBMX's behavioral effects are due to increases in NE neurotransmission. These results are similar to those obtained with true opiate withdrawal in rats, strengthening the idea that suppression of operant behavior by IBMX involves mechanisms in common with opiate withdrawal. It may be a useful way of objectively studying the expression of the withdrawal syndrome in the absence of opiates and/or a way of determining if a drug can selectively block withdrawal.

3-Isobutyl-1-methylxanthine IBMX Clonidine Opiate-withdrawal Rats

THE opiate withdrawal syndrome consists of a variety of autonomic and behavioral signs, including diarrhea, urination, and irritability, which are reduced by administration of opiates. Nonopiates, such as clonidine, also alleviate many signs of opiate withdrawal [8, 24, 25, 35, 43], indicating that the expression of withdrawal may be secondary and distinct from the initial opiate action or the adaptive process (i.e., dependence). Further evidence for this separation is the demonstration of a quasi-morphine withdrawal syndrome (QMWS), produced in opiate naive rats treated with methylxanthines alone or in combination with the opiate antagonist naloxone [9,10]. Examination of the QMWS may therefore offer a method of defining the role of specific neurotransmitters in either the development or expression of opiate withdrawal, and a means by which effective nonopiate drugs, with fewer undesirable side effects than clonidine, may be discovered for use in opiate detoxification programs.

Numerous studies implicate a role of the noradrenergic nucleus locus coeruleus (LC), the major NE containing nucleus in the brain, in the opiate withdrawal syndrome [1, 11, 33, 48], with the principal evidence being that NE turnover and levels of the major NE metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), are increased during morphine tolerance and further increased during withdrawal in rats. The increase in NE activity during withdrawal can be reversed by administration of the α_2 agonist clonidine [11,41], which also alleviates naloxone precipitated morphine withdrawal in chronically [43] or acutely [16] dependent rats. Clonidine is a potent central acting α-adrenergic agonist, widely used as an antihypertensive agent, which stimulates α_2 receptors in the LC resulting in a decrease in noradrenergic activity, measured both by firing rate [7,45] and neurotransmitter release and synthesis [46]. Electrophysiological studies in rats undergoing naloxone precipitated withdrawal have shown an increase in firing of neurons in the LC [1]. Clonidine is believed to reduce firing of LC neurons via stimulation of α_2 (auto) receptors [46], although higher doses stimulate post-synaptic α_1 receptors [2]. Consequently, a reduction in brain levels of MHPG is observed [45], since synthesis of NE is apparently coupled to neuronal activity [31,32], which in turn may be reduced via both mechanisms.

Although a role of NE in opiate withdrawal seems to be well established, the involvement of NE in the QMWS has

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[3]Supported in part by USPHS Grant DA 01880.

only recently been studied. Administration of isobutylmethylxanthine (IBMX) to rats produces increases in central nervous system catecholamine utilization consistent with that found during naloxone precipitated withdrawal (e.g., increased norepinephrine turnover [33]), as well as an increase in serotonin metabolism [4]. The noradrenergic effects of IBMX have been examined [21, 22, 40], with the goal of understanding the involvement of central NE in anxiety and the opiate withdrawal syndrome. The QMWS induced by methylxanthines may resemble opiate withdrawal due to central noradrenergic hyperactivity. Indeed, the LC could be the central link between clinical anxiety and the expression of many withdrawal signs, particularly those which are psychogenic and autonomic in origin.

The QMWS produced by IBMX and naloxone can be reversed by concurrent administration of clonidine (50 μg/kg) and intravenous administration of IBMX stimulates LC impulse flow in a clonidine reversible fashion [27]. Examination of MHPG levels in several brain regions containing LC projections (e.g., hippocampus and cerebellum) showed that clonidine (25–75 μg/kg) reversed the increase in MHPG concentration produced by IBMX (37–100 μM/kg) [21]. This effect of clonidine does not appear to be simply due to a decrease in LC firing since the benzodiazepine, diazepam, which also lowers LC activity [26], did not reverse IBMX's effect upon MHPG. Interestingly, naloxone (1 mg/kg) did not further increase MHPG levels when given in combination with a high dose of IBMX (100 μM/kg), either because the increase due to IBMX is already maximal (175%) or the exacerbation of the behavioral symptoms does not invove the noradrenergic system exclusively.

Clonidine alleviates naloxone precipitated suppression of FR behavior in rats previously administered morphine [8, 17, 44]. Administration of d-amphetamine in combination with the opiate antagonist naltrexone likewise induces an altered state akin to a morphine withdrawal syndrome which is attenuated by clonidine [18]. These studies indicate that if the operant behavioral effects produced by low doses of IBMX can be considered a model of the QMWS, then clonidine should attenuate such effects, just as it does this very sensitive [23] measure of opiate withdrawal. IBMX was therefore studied because recent neurochemical and unconditioned behavioral data indicate that IBMX may act through a mechanism similar to that involved in the expression of true opiate withdrawal [21, 22, 39].

It was first necessary to obtain data regarding doses which affected rats responding for food reinforcement on a fixed ratio schedule. Similar operant behavioral effects of clonidine have been studied recently [16], with selective effects at α_2 receptors demonstrable with only relatively low doses. Although doses of clonidine greater than 100 μg/kg appear to affect other neurotransmitter systems such as dopamine [36], or purines [30], a selective behavioral action at α_2 receptors can be obtained with doses under 30 μg/kg [16]. These lower doses were used to attempt to antagonize the operant behavioral effects of IBMX.

METHOD

Animals

Adult male Long-Evans rats, obtained from the Blue Spruce Farms (Altamont, NY) and weighing 400–450 g (free-feeding) were used. Rats were individually housed in hanging, stainless steel cages (H17 × W17 × L25 cm) in a room maintained at 21±1°C temperature, 40–50% relative humidity, a light-dark cycle of 12 hr (light on from 0700–1900 hr), and water available ad lib. Subjects were approximately 4 months of age when body weights were gradually reduced to 80% of free-feeding and operant training began.

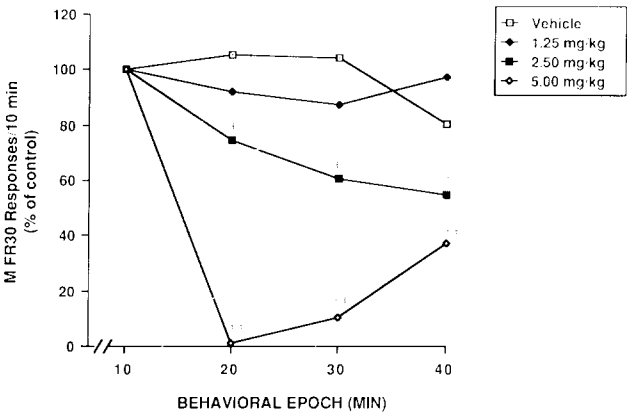

FIG. 1. Effect of IBMX administered 10 min after the start of an FR30 operant behavioral session. Data (n=5) are expressed as the number of FR30 responses following IBMX administration as a percent of responses during the 10 min epoch prior to drug. $*p<0.05$ and $**p<0.01$ vs. 0 IBMX control using Dunnett's test.

Apparatus and Procedure

Operant behavioral sessions took place in standard conditioning chambers (Model No. 143-22; BRS/LVE, Beltsville, MD). The operant chambers were enclosed in custom built insulated environmental cubicles, equipped with closed-circuit video cameras and speakers for introduction of masking noise [43]. Chambers were equipped with a lever which could be depressed by a weight of 20 to 25 g. A house light and cue light above the lever were illuminated during the behavioral session, signaling availability of reinforcement. Food pellets weighing 45 mg (formulation T101, Bioserv, Frenchtown, NJ) were dispensed upon completion, by the rat, of the required number of responses. Behavior sessions were controlled by a custom made microcomputer interface for TRS-80® Color Computers (Tandy Radio Shack, Fort Worth, TX). The interface provided power supply and white masking noise for the chambers while monitoring inputs and controlling outputs to chamber lights and food dispenser. Timing and monitoring of behavioral events was provided by a 60 Hz interrupt-driven machine language routine.

Data accumulated by the Color Computer were transferred to tape cassettes at the end of the behavioral sessions and printouts were obtained for later analyses. The data were comprised of the total number of responses emitted, number of reinforcers delivered to the rat, and responses/min for the entire session. After the pilot studies with IBMX it was determined that drug could be administered during the behavioral sessions, since the onset of action was rapid, within 1–2 min, and recovery of behavior was often observed during the session. Thus, data collected during 10 min epochs of the 40 min were compared to the 10 min pre-IBMX epoch.

Experiments began after stable responding during 30 min daily sessions was obtained and rats were habituated to saline (1 mg/kg) and IBMX vehicle injections (1 or 2 ml/kg) prior to the behavioral session. Stability was defined as a

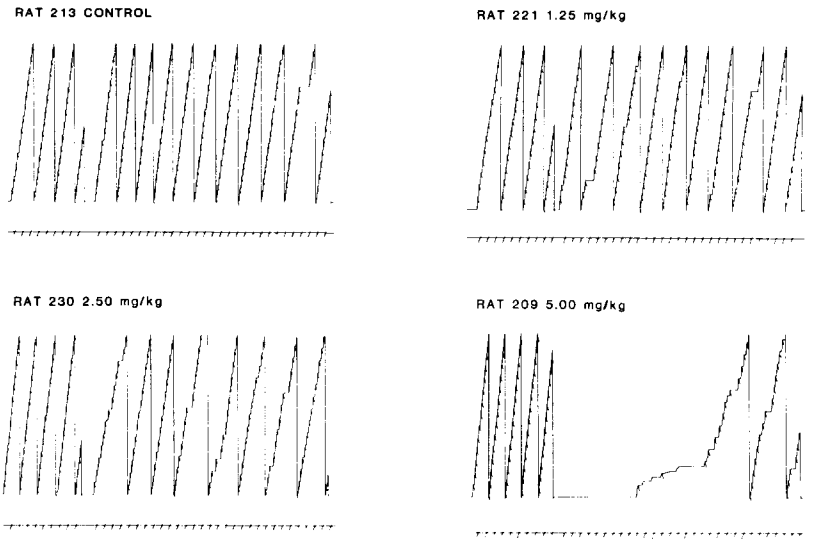

FIG. 2. Cumulative response records for representative animals illustrating the behavioral effect of IBMX (0, 1.25, 2.50, and 5.00 mg/kg). IBMX or vehicle (2% Pluronic® F68, 2 ml/kg) was administered during the 1 min period, 10 min after the start of the behavioral session. The stepping pen reset after 400 responses on the lever. Downward strokes of the stepping pen indicate delivery of a food pellet. Downward strokes of the event pen indicate 1 min intervals.

coefficient of variation less than 10% during three consecutive sessions. This procedure allowed the use of previous sessions, when saline or drug vehicle (2% Pluronic F68 polyol) was administered, to be used for comparison with drug sessions. The drug session was preceded by at least 2 drug-free sessions such that at least 72 hours had passed between exposures to IBMX.

Effect of IBMX on Behavior

In pilot experiments to determine doses which affected operant responding, IBMX (0.625 to 30.0 mg/kg) was administered intraperitoneally 30 min prior to the behavioral session. The peak effect was very early and, at higher doses, complete recovery did not occur for at least 3 hours. Doses based upon neurochemical studies (100 μM/kg; 22 mg/kg [21,40]) were too high to administer 30 min prior to the behavioral sessions, producing complete suppression of responding, profuse salivation and occasional convulsions. Since clonidine itself may be behaviorally active at the doses which would antagonize IBMX, it appeared to be necessary to take this into account. A protocol which allowed examination of both the behavioral effect of clonidine, as well as its interaction with IBMX, was to administer IBMX 10 min after the start of the session and use the first 10 min to observe the effect of the pretreatment (e.g., clonidine) alone. The rats used in the two previous experiments were trained to perform during a 40 min session with a 1 min time out 10 min after the beginning of the session. The 1 min period, during which house and cue lamps were turned off and there were no programmed consequences of responding, allowed injection of either vehicle or IBMX. This period imposed a uniform drug injection procedure and also allowed time for initial absorption of drug before the session resumed. The session was thus divided into 4 epochs of 10 min duration with responses being summed for each epoch and compared to the same epoch during the previous control day, as well as with the initial 10 min epoch of that day. Rats were habituated to injections of vehicle during the operant sessions and randomly assigned to one of four groups (n=5), balanced for response rates, and given four doses of drug (0, 1.25, 2.5, or 5.0 mg IBMX/kg). At least 72 hr had elapsed between previous exposures to IBMX.

Effects of Clonidine and IBMX

The rats were randomly assigned to 4 groups receiving clonidine (0, 5, 15, and 30 μg/kg; n=5) 30 min before the session. During the session, 5 mg IBMX/kg was administered. The groups were balanced for individual response rates and at least 72 hours had elapsed since previous exposure to IBMX.

Analysis of Results

Subjects were assigned to drug treatment groups following a stratified random procedure based upon response rates obtained during the control day in which vehicle was administered. Data were expressed as a percent of the corresponding previous control session, or in the experiment with IBMX administered during the session, expressed as a percentage of responding during the 10 min epoch, before drug administration on that day. These steps reduce the variability due to individual differences in response rates. The data were analyzed by repeated measures ANOVA and individual comparisons were made using Duncan's and Dunnett's procedures, when appropriate, with a $p<0.05$ deemed significant [47]. A least-squares linear regression analysis was made for dose-response data. Error terms in repeated measure designs were pooled when significant interactions were obtained (e.g., indicating that differences between groups occurred at a particular epoch during the session) and a Satterthwaite correction for pooled error degrees of freedom was calculated [47].

Drugs

Clonidine HCl was a gift from Boehringer Ingleheim Ltd. (Elmsford, NY). Doses of clonidine refer to the salt and were dissolved daily in saline and administered IP in a volume of 1 ml/kg. IBMX (Sigma Chemical Co., St. Louis, MO) was also made fresh daily as a suspension in 2% Pluronic® F68 (BASF Wyandotte Corp., Wyandotte, MI) which was sonicated prior to injection at 2 ml/kg (IP).

RESULTS

Effect of IBMX on Operant Behavior

A repeated measures ANOVA showed that IBMX produced a significant suppression of responding, $F(3,15)=5.62$, $p<0.009$, Fig. 1. When the data are averaged over the entire 30 min period following administration, the 1.25 mg IBMX/kg dose did not significantly affect responding, but 2.5 and 5.0 mg IBMX/kg suppressed behavior to 63.1±13.5 and 16.2±8.2 (mean±SEM) % of control, respectively. Figure 2 shows individual cumulative response records illustrating the behavioral effect of IBMX administered 10 min after the start of the 40 min FR30 operant session. All of the rats show a steady rate of responding prior to drug or vehicle; rats treated with vehicle continue to respond at a steady rate until the latter part of the session when satiation probably starts to occur and responding rates diminish slightly. Administration of 5 mg IBMX/kg caused an immediate cessation of responding, with recovery starting to occur during the latter part of the session, whereas the 2.5 mg/kg dose suppressed running rates without totally disrupting responding.

Clonidine and IBMX

The means for each of the clonidine dose groups are shown in Fig. 3. A repeated measures ANOVA did not reveal a main effect of clonidine, $F(3,16)=1.24$, $p<0.33$, but a significant interaction between clonidine and behavioral epoch emerged, $F(9,48)=5.46$, $p<0.001$. An analysis of simple main effects showed that a significant effect of clonidine appeared prior to IBMX administration (i.e., 10 min into the session), but not at subsequent time periods, $F(3,37)=8.92$, $p<0.001$. Subsequent comparisons using Dunnett's analysis indicated that all doses of clonidine reduced the number of responses made during the initial 10 min epoch. The highest dose, 30 μg/kg, suppressed FR30 responding to 36.6±5.6% of control; the 15 and 5 μg/kg doses produced response rates of 61.6±10.0 and 92.2±3.2% of control, respectively (Fig. 3). A log-linear regression analysis of the effect of clonidine on operant responding during the first 10 min epoch revealed a correlation coefficient of −0.997 ($p<0.001$). The ED_{50} computed from this analysis was 20.3 μg/kg. The ability of clonidine to antagonize the suppression of responding produced by IBMX is shown in Fig. 3 as well. Although the lowest dose of clonidine, as did other doses, decreased responding in the first epoch, it also significantly antagonized the effect of IBMX during all subsequent epochs ($p<0.01$, Dunnett's test). The higher doses of clonidine also significantly antagonized IBMX but only at later epochs. Figure 4 shows cumulative records illustrating the effects of clonidine against IBMX's effect upon FR30 responding. The higher doses of clonidine (15 and 30 μg/kg) clearly dramatically suppressed responding during the 10 min prior to IBMX administration. IBMX administration produced an immediate suppression of responding, all doses of clonidine partially antagonized the IBMX-induced suppression during the subsequent 30 min.

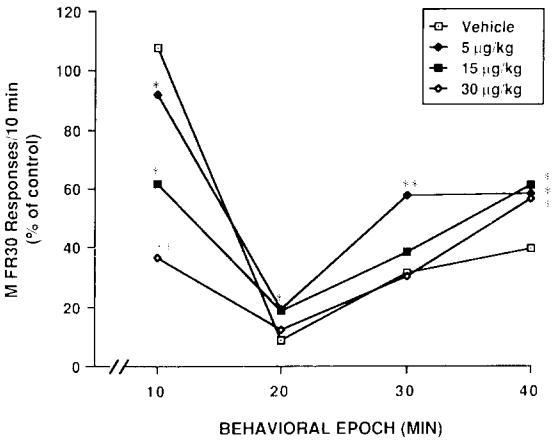

FIG. 3. Partial attenuation of the behavioral suppression of IBMX by clonidine. Clonidine was administered 30 min prior to and IBMX (5 mg/kg IP) was administered 10 min after the beginning of the 40 min FR30 session. Data (n=5) are expressed as the number of responses during the session following drug as a percent of the corresponding epoch during the control day in which vehicles (0.9% saline and 2% Pluronic® F68) were administered. *$p<0.05$ and **$p<0.01$ vs. 0 clonidine control using Dunnett's test.

DISCUSSION

The purpose of the present series of experiments was to determine if operant behavior could be used to study the pharmacological actions of IBMX. Since another goal of these investigations was to develop a paradigm which is sensitive to the QMWS produced by IBMX, the operant behavioral effects of doses of IBMX under 10 mg/kg were examined. Doses of IBMX higher than 10 mg/kg administered before the operant sessions produced complete suppression of FR30 operant behavior, with doses of 15 and 30 mg/kg causing salivation and convulsions in several subjects. However, the 10 mg/kg dose did not produce "classical" signs of the QMWS when administered IP.

Even in studies of the QMWS syndrome, low doses of IBMX do not reliably produce signs of opiate withdrawal. For example, 1 hr after SC administration of 10 mg IBMX/kg, 6 of 14 signs of QMWS were observed: ptosis, rearing, restlessness, body shakes, head shakes, and diarrhea [19]. The increase in these signs was less than 50%, with the incidence of diarrhea (which was not observed by us) at 33% following such doses of IBMX [19]. It would be difficult to observe a relatively small increase in such signs with the sample size (n=5–7) typically used in operant experiments. This comparison points to the utility of using an objective analysis of behavior where nearly complete suppression of behavior occurs at a dose which might produce a very small increase in quantal signs, indicating that operant behavior is more sensitive as a measure of QMWS. However, it is difficult to compare results of studies using different routes of administration (SC vs. IP). IBMX is reported to be 5–6 times more potent than theophylline and caffeine in producing the QMWS [6]. Studies of caffeine and theophylline using operant behavior [12, 29, 34] indicate that doses of caffeine higher than 25 mg/kg should have rate-decreasing effects. The results using FR30 operant behavior indicate that IBMX

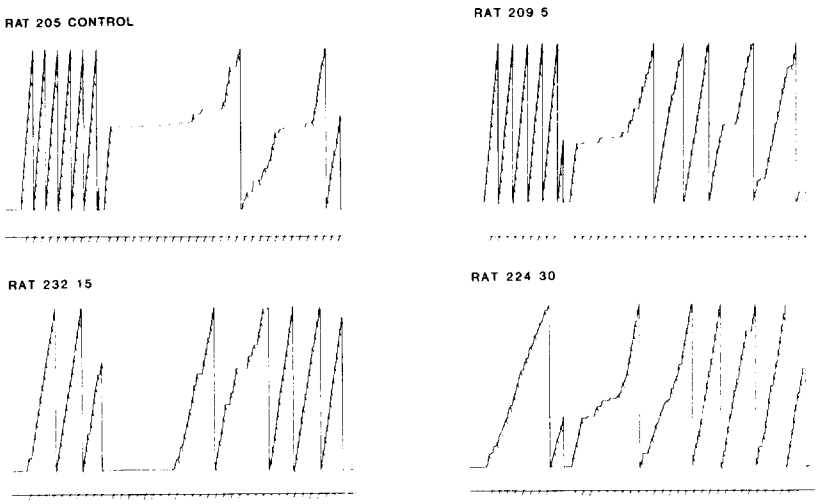

FIG. 4. Cumulative response records illustrating the behavioral effect of clonidine and the attenuation of IBMX's effect upon fixed ratio behavior. Clonidine (0, 5, 15, or 30 μg/kg) was administered 30 min before the operant session, and IBMX (5.0 mg/kg) was administered during the 1 min period, 10 min after the start of the behavioral session. See Fig. 2 for additional explanations of cumulative records.

produced significant suppression of behavior with doses as small as 2.5 mg/kg administered during 40 min FR30 sessions, indicating that IBMX may be up to 10 times more potent than caffeine in suppressing FR operant responding.

Operant behavior has been used to measure morphine withdrawal in rats and its attenuation by clonidine (1–50 μg/kg) [8, 17, 35, 44]. In those studies, optimal attenuation of naloxone precipitated suppression of operant behavior and body weight loss occurs at lower doses of clonidine (1–10 μg/kg) with higher doses (e.g., 50 μg/kg) resulting in less apparent attenuation, probably because of intrinsic behavioral and diuretic actions of clonidine. Nonetheless, clinical studies show that clonidine relieves most signs and symptoms of opiate withdrawal [24,25], verifying that an operant behavioral model can be used to predict the clinical efficacy of drugs to attenuate opiate withdrawal.

Clonidine, at low doses, as used in the present studies, is relatively selective toward the α_2 (auto) receptor. The α_2 antagonist yohimbine completely antagonizes the operant behavioral suppression caused by such low doses of clonidine [16]. These data suggested that the low doses of clonidine used herein suppressed behavior through a selective α_2 receptor agonist property. At higher doses (e.g., 90 μg/kg) clonidine may lose selectivity and affect several other receptor populations, including cholinergic [13, 14, 37], histaminergic [3], purinergic [30] and α_1 noradrenergic [2]. The ED_{50} for suppression of FR15 responding obtained in previous experiments [16] was 25 μg/kg compared with 20.3 μg/kg obtained using FR30 behavior, demonstrating the reliability and reproducibility of these effects.

The 5 μg/kg dose of clonidine, which marginally suppressed operant responding, significantly attenuated the behavioral effect of IBMX. The 30 μg/kg dose was not more effective in reducing behavioral suppression caused by IBMX, perhaps owing to nonspecific effects of IBMX, or even to clonidine's action at postsynaptic or multiple sites at the higher doses. The latter possibility is unlikely since the behavioral effects of IBMX were not additive with the higher dose of clonidine and some attenuation is still evident. Interestingly, examination of the cumulative records of Fig. 4 gives some idea of the conservative nature of the way the data were analyzed for attenuating effects of clonidine and the complex nature of the interaction of IBMX with clonidine. One might interpret the diminished effects of IBMX in clonidine pretreated subjects as a consequence of a lowered baseline (1st 10 min) or evidence for a rate-dependent effect [42]. The lower the initial response rates, the smaller the relative depressing effect of 5.0 mg IBMX/kg. However, viewed from a different perspective, if the 1st 10 min epoch is used as the control rate, the higher doses of clonidine pretreatment appear to block even more effectively the behavioral effects of IBMX. At 15 μg/kg and 30 μg/kg, clonidine pretreated subjects respond at about 67% and 125% of pre-IBMX rates, respectively.

Although it has been assumed that clonidine attenuates the opiate withdrawal syndrome via actions at the LC, it is possible that clonidine also acts at other important sites in the CNS. Clonidine (25 μg/kg) depresses electrically evoked preganglionic sympathetic discharges in spinally transected cats [20]. These data suggest that clonidine suppresses intraspinal and spinal reflex pathways which could be hyperactive during opiate withdrawal. Since opiates also depress preganglionic sympathetic activity [20], it is possible that hyperactivity does result after the development of dependence and preganglionic neurons in the spinal cord, in addition to rostral sites, are involved in the autonomic signs of withdrawal. It has also been proposed that projections of the LC are associated with physiological correlates of anxiety [38]. These projections include hypothalamic, medullary, and spinal sympathetic areas, which mediate the tachycardia, piloerection, gastrointestinal hypermotility, urination, and diarrhea often observed during anxiety and opiate withdrawal. It has been suggested [5] that suppression by clonidine and guanfacine of the hypertensive response to naloxone in morphine dependent rats is consistent with clinical antiwithdrawal actions. Thus, blockade of autonomic symptoms of opiate withdrawal abstinence mediated by rostral and/or spinal α_2 adrenoceptors may depress the physiological consequences of withdrawal.

REFERENCES

1. Aghajanian, G. K. Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine. *Nature* **276:** 186–188, 1978.
2. Anden, N. E., H. Corrodi, K. Fuxe, B. Hokfelt, T. Hokfelt, C. Tydin and T. Svensson. Evidence for a central noradrenaline receptor stimulation by clonidine. *Life Sci* **9:** 513–523, 1970.
3. Anden, N. E. and M. Grabowska-Anden. Influence of the H_2-receptor blocking agent mitiamide on the clonidine-induced changes in brain catecholamine turnover. *J Neural Transm* **47:** 175–180, 1980.
4. Berkowitz, B. A. and S. Spector. The effect of caffeine and theophylline on the disposition of brain serotonin in the rat. *Eur J Pharmacol* **16:** 322–325, 1971.
5. Buccafusco, J. J., D. C. Marshall and R. M. Turner. A comparison of the inhibitory effects of clonidine and guanfacine on the behavioral and autonomic components of morphine withdrawal in rats. *Life Sci* **35:** 1401–1408, 1984.
6. Butt, N. M., H. O. J. Collier, N. J. Cuthbert, D. L. Francis and S. A. Saeed. Mechanism of quasi-morphine withdrawal behaviour induced by methylxanthines. *Eur J Pharmacol* **53:** 375–378, 1979.
7. Cedarbaum, J. M. and G. K. Aghajanian. Catecholamine receptors on locus coeruleus neurons: Pharmacological characterization. *Eur J Pharmacol* **44:** 375–385, 1977.
8. Colelli, B., D. R. Meyer and S. B. Sparber. Clonidine antagonizes disruption of fixed ratio operant behavior in morphine pelleted rats given naloxone. *Pharmacologist* **18:** 236, 1976.
9. Collier, H. O. J., N. J. Cuthbert and D. L. Francis. Character and meaning of quasi-morphine withdrawal phenomena elicited by methylxanthines. *Fed Proc* **40:** 1513–1518, 1981.
10. Collier, H. O. J., D. L. Francis, G. Henderson and C. Schneider. Quasi-morphine abstinence syndrome. *Nature* **249:** 471–473, 1974.
11. Crawley, J. N., R. Laverty and R. H. Roth. Clonidine reversal of increased norepinephrine metabolite levels during morphine withdrawal. *Eur J Pharmacol* **57:** 247–250, 1981.
12. Davis, T. R. A., C. J. Kensler and P. B. Dews. Comparison of behavioral effects of nicotine, d-amphetamine, caffeine and dimethylheptyltetrahydrocannibinol in squirrel monkeys. *Psychopharmacologia* **32:** 51–65, 1973.
13. Delbarre, B. and H. Schmitt. Sedative effects of α-sympathomimetic drugs and their antagonism by adrenergic and cholinergic blocking drugs. *Eur J Pharmacol* **13:** 356–363, 1971.
14. Delbarre, B. and H. Schmitt. Effects of clonidine and some α-adrenoceptor blocking agents on avoidance conditioned reflexes in rats: Their interactions and antagonism by atropine. *Psychopharmacologia* **35:** 195–202, 1974.
15. Delini-Stula, A., P. Baumann and O. Buch. Depression of exploratory activity by clonidine in rats as a model for the detection of relative pre- and postsynaptic central noradrenergic receptor selectivity of α-adrenolytic drugs. *Naunyn Schmiedebergs Arch Pharmacol* **307:** 115–122, 1979.
16. Dwoskin, L. P. and S. B. Sparber. Comparison of yohimbine, mianserin, chlorpromazine and prazosin as antagonists of the suppressant effect of clonidine on operant behavior. *J Pharmacol Exp Ther* **226:** 57–64, 1983.
17. Dwoskin, L. P., B. S. Neal and S. B. Sparber. Yohimbine exacerbates and clonidine attenuates acute morphine withdrawal in rats. *Eur J Pharmacol* **90:** 269–273, 1983.
18. Fossum, L. H. and S. B. Sparber. Potentiation by naltrexone of naltrexone of d-amphetamine-induced behavioral suppression and its reversal by clonidine. *Life Sci* **31:** 2827–2835, 1982.
19. Francis, D. L., A. C. Roy and H. O. J. Collier. Morphine abstinence and quasi-abstinence effects after phosphodiesterase inhibitors and naloxone. *Life Sci* **16:** 1901–1906, 1975.
20. Franz, D. N., B. D. Hare and K. L. McCloskey. Spinal sympathetic neurons: Possible sites of opiate-withdrawal suppression by clonidine. *Science* **215:** 1643–1645, 1982.
21. Galloway, M. P. and R. H. Roth. Clonidine prevents methylxanthine stimulation of norepinephrine metabolism in rat brain. *J Neurochem* **40:** 246–251, 1983.
22. Galloway, M. P. and R. H. Roth. Neuropharmacology of 3-isobutylmethyl-xanthine: Effects on central noradrenergic systems *in vivo*. *J Pharmacol Exp Ther* **227:** 1–8, 1983.
23. Gellert, V. and S. B. Sparber. A comparison of the effects of naloxone upon body weight loss and suppression of fixed-ratio operant behavior in morphine-dependent rats. *J Pharmacol Exp Ther* **201:** 44–54, 1977.
24. Gold, M. S., D. E. Redmond and H. D. Kleber. Clonidine blocks acute opiate-withdrawal symptoms. *Lancet* **1:** 599–602, 1978.
25. Gold, M. S., D. E. Redmond and H. D. Kleber. Clonidine in opiate withdrawal. *Lancet* **1:** 929–930, 1978.
26. Grant, S. J., Y. H. Huang and D. E. Redmond. Benzodiazepines attenuate single unit activity in the locus coeruleus. *Life Sci* **27:** 2231–2236, 1980.
27. Grant, S. J. and D. E. Redmond. Clonidine suppresses methylxanthine induced quasi-morphine withdrawal syndrome. *Pharmacol Biochem Behav* **17:** 655–658, 1982.
28. Grant, S. J. and D. E. Redmond. Methylxanthine activation of noradrenergic unit activity and reversal by clonidine. *Eur J Pharmacol* **85:** 105–109, 1982.
29. Harris, R. A., D. Snell and H. H. Loh. Effects of stimulants, anorectics, and related drugs on schedule-controlled behavior. *Psychopharmacology (Berlin)* **56:** 49–55, 1978.
30. Katsuragi, T., L. Kuratomi and T. Furukawa. Clonidine-evoked selective P_1-purinoceptor antagonism of contraction of guinea-pig urinary bladder. *Eur J Pharmacol* **121:** 119–122, 1986.
31. Korf, J., G. K. Aghajanian and R. H. Roth. Increased turnover of norepinephrine in the rat cerebral cortex during stress: Role of the locus coeruleus. *Neuropharmacology* **12:** 933–938, 1973.
32. Korf, J., G. Aghajanian and R. H. Roth. Stimulation and destruction of the locus coeruleus: Opposite effects on 3-methyoxy-4-hydroxyphenyl-glycol sulfate levels in the rat cerebral cortex. *Eur J Pharmacol* **21:** 305–310, 1973.
33. Laverty, R. and R. H. Roth. Clonidine reverses the increased norepinephrine turnover during morphine withdrawal in rats. *Brain Res* **182:** 482–485, 1980.
34. Meliska, C. T. and R. E. Brown. Effects of caffeine on schedule controlled responding in the rat. *Pharmacol Biochem Behav* **16:** 745–750, 1982.
35. Meyer, D. R. and S. B. Sparber. Clonidine antagonizes body weight loss and other symptoms used to measure withdrawal in morphine pelleted rats given naloxone. *Pharmacologist* **18:** 236, 1976.
36. Ng, J., E. L. Phelan, D. D. McGregor, R. Laverty, K. M. Traylor and H. Smirk. Properties of catapress, a new hypotensive drug: A preliminary report. *NZ Med J* **66:** 864–870, 1967.
37. Paalzow, G. and L. Paalzow. Clonidine antinociceptive activity: Effects of drugs influencing central monoaminergic and cholinergic mechanisms in the rat. *Naunyn Schmiedebergs Arch Pharmacol* **292:** 119–126, 1976.
38. Redmond, D. E. and Y. H. Huang. New evidence for a locus coeruleus-norepinephrine connection with anxiety. *Life Sci* **25:** 2149–2162, 1979.
39. Reinhard, J. F., Jr. and R. H. Roth. Noradrenergic modulation of serotonin synthesis and metabolism. I. Inhibition by clonidine *in vivo*. *J Pharmacol Exp Ther* **221:** 541–546, 1981.
40. Reinhard, J. F., Jr., M. P. Galloway and R. H. Roth. Noradrenergic modulation of serotonin synthesis and metabolism. II. Stimulation by 3-isobutyl-1-methylxanthine. *J Pharmacol Exp Ther* **226:** 764–769, 1983.
41. Roth, R. H., J. D. Elsworth and D. E. Redmond. Clonidine suppression of noradrenergic hyperactivity during morphine withdrawal by clonidine: Biochemical studies in rodents and primates. *J Clin Psychiatry* **43:** 42–46, 1982.

42. Sanger, D. J. and D. E. Blackman. Rate-dependent effects of drugs: A review of the literature. *Pharmacol Biochem Behav* **4:** 73–83, 1976.
43. Sparber, S. B. Use of learned behavior in testing for neurotoxicity. In: *The Effects of Foods and Drugs on the Development and Function of the Nervous System,* edited by R. M. Gryder and V. H. Frankos. Washington, DC: HHS Publication (FDA), 1980, pp. 80–107.
44. Sparber, S. B. and D. R. Meyer. Clonidine antagonizes naloxone induced suppression of conditioned behavior and body weight loss in morphine dependent rats. *Pharmacol Biochem Behav* **9:** 319–325, 1978.
45. Starke, K. Regulation of noradrenaline release by presynaptic receptor systems. *Rev Physiol Biochem Pharmacol* **77:** 1–124, 1977.
46. Starke, K. and H. Montel. Involvement of α-receptors in clonidine-induced inhibition of transmitter release from central monoamine neurones. *Neuropharmacology* **12:** 1073–1080, 1973.
47. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1971.
48. Zigon, J. R., M. J. Bannon and R. H. Roth. Comparison of two α-noradrenergic agonists (clonidine and guanfacine) on norepinephrine turnover in the cortex of rats during morphine abstinence. *Eur J Pharmacol* **70:** 565–570, 1981.