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Short communication

Effect of alprazolam on opiate withdrawal: a combined behavioural and microdialysis study

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Abstract

Benzodiazepines are commonly abused concurrently with opioids. The pharmacological rationale for this remains unknown. The present study has addressed behaviourally and neurochemically the action of alprazolam on the naloxone-precipitated morphine withdrawal syndrome in male rats. In naloxone (1 mg kg^{-1} i.p.)-precipitated morphine withdrawn rats, alprazolam (2.5 mg kg^{-1} s.c.) reduced the severity of the affective component, as measured by squeal on touch hostility, and the physical sequelae of opioid withdrawal. The microdialysis study in anaesthetized rats identified an increase in noradrenaline levels in hippocampal dialysates in rats undergoing naloxone-precipitated opioid withdrawal. Acute treatment with alprazolam (2.5 mg kg^{-1} s.c.) 20 min before administration of naloxone prevented the previously identified increase in noradrenaline in hippocampal dialysates. The only observable effect alprazolam induced in non-morphine-dependent rats was a 15% reduction in spontaneous locomotor activity. In conclusion, one interpretation of the data suggests that alprazolam decreases the withdrawal syndrome in rats through dampening down the previously identified hyperactivity of the locus coeruleus.

Keywords: Morphine withdrawal; Benzodiazepine; Microdialysis

1. Introduction

The opiate withdrawal syndrome in animals has been shown to involve increased central noradrenergic activity. For example Aghajanian (1978) has shown that following chronic morphine treatment in rats, naloxone produces a dramatic increase in the firing rate of the locus coeruleus neurones. Furthermore, it has been suggested that the increased activity of the locus coeruleus, a brainstem nucleus containing high concentrations of noradrenaline, mediates many of the somatic signs of opiate withdrawal (Maldonado and Koob, 1993). Recent pharmacotherapeutic approaches to opiate withdrawal have centered on the locus coeruleus as a possible site of therapeutic efficacy. That is, clonidine possesses a demonstrated utility against the opiate withdrawal syndrome in man (Gold et al., 1978). Using in vivo brain microdialysis, Sharp and colleagues (Done et al., 1992) found that the efflux of noradrenaline increased in the major terminal projection region of the locus coeruleus, the ventral hippocampus, following naloxone-precipitated morphine withdrawal in the rat. In addition, this increase in noradrenaline output was attenuated by clonidine (Done et al., 1992). Furthermore, Coupar (1992) has shown the capacity of a range of α_2-adrenoceptor agonists to reduce many of the somatic signs of opioid withdrawal in rats.

In man, several benzodiazepine compounds have been used to attenuate the symptoms of opiate withdrawal (Sugerman et al., 1971; Drummond et al., 1989). However, the abuse of benzodiazepines, in particular the concurrent use of benzodiazepines with other drugs of abuse, is well documented (Darke, 1994). An emerging clinical picture when opiate addicts abuse benzodiazepines concurrently with their opiate, is one of greater psychopathology and increased morbidity (Darke et al., 1993). Although anecdotal evidence suggests that amphetamine/ecstacy users take benzodiazepines to 'come-down', the pharmacological mechanism responsible for the use of benzodiazepines with opiates is unknown. Indeed, the mechanism through

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which benzodiazepines decrease the withdrawal phenomenon from opiates remains unclear. Thus, whilst benzodiazepines reduce the severity of the withdrawal response, it is unlikely that this is the main reason for their concurrent use with opiates.

In order to identify the effects of benzodiazepines on the expression of opiate abstinence, this study has investigated the effect of alprazolam on the physical withdrawal syndrome and on the naloxone-precipitated increase in the release of noradrenaline in the hippocampus of the morphine-dependent rat.

2. Materials and methods

2.1. Animals and induction of opiate dependence

Adult male Sprague-Dawley rats (250–310 g; Harlan Olac, Bicester, UK) were housed in cages of 6, on a 12 h light/dark cycle and allowed food and water ad libitum. Morphine dependence was induced by twice daily (i.p.) injections of morphine at 08:00 and 20:00 h using an increasing schedule of morphine. The initial dose on the first day was 2.5 mg kg^{-1} (i.p., b.d.) increasing to 5, 10, 60, 80, 100, 100, 100 mg kg^{-1} each successive day, until a dose of 100 mg kg^{-1} (i.p., b.d.) was achieved on the eighth day (modified from Ohnishi et al., 1989). The control rats received 8 twice daily injections of vehicle.

2.2. Assessment of opiate dependence

Animals were transferred in pairs (one treatment and one control) to an observation chamber where withdrawal was precipitated by injection of naloxone (1 mg kg^{-1} i.p.) and abstinence signs recorded in 5 min epochs for a 30 min period post-naloxone on day 8. Additionally, weight and core body temperature were monitored every 30 min for the 2 h post-naloxone treatment.

The following signs were recorded on a scored scale (+, mild; ++, moderate; +++, intense; ++++, severe): squeal on touch, salivation, diarrhoea, urination, rhinorrhoea, ptosis. Episodes of wet dog shakes, writhing, burrowing, teeth chattering (vacuous chewing), jumping, escape behaviour, forepaw treading and tremor, and head twitching were counted (i.e. recorded quantitatively). Rectal temperature was recorded using a thermistor probe coupled to a Pan Lab digital thermometer immediately before and at 30, 60 and 120 min after naloxone.

2.3. Microdialysis procedure

Each rat ($n = 6$) was anaesthetized with chloral hydrate (400 mg kg^{-1} i.p.) and placed in a Kopf stereotaxic instrument with the incisor bar set a zero with respect to the interaural line. The skull was exposed and a small hole was drilled so that a microdialysis probe with a 5 mm exposed dialysis fibre tip (Gambro membrane, regenerated cellulose-like material o.d. 200 μM) may be implanted in the ventral hippocampus relative to bregma according to the following coordinates, AP, −0.4; L, −4.6; DV, −9.5; Paxinos and Watson (1986). The probe was perfused continuously at 1.2 μl min^{-1} with an artificial cerebrospinal fluid containing: NaCl 140.0 mM, KCl 3.0 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.0 mM, Na_2HPO_4 1.2 mM, NaH_2PO_4 0.27 mM, glucose 7.2 mM, pH 7.4. In addition, 5.0 μM of desipramine hydrochloride was also added to increase the basal levels of noradrenaline. After 2 h had elapsed following the implantation of the dialysis probe, perfusates were collected every 20 min continuously for 2 h and 20 min.

2.4. Microdialysis experimental protocol

After the implantation of the dialysis probe, the output of noradrenaline stabilised over a 2–3 h baseline period: the effect of drug challenge was followed for a further 140 min. Naloxone (1 mg kg^{-1} i.p.) was administered to each treatment group: morphine-naive and repeated morphine group, 3 h after the last injection of chronic morphine (100 mg kg^{-1} s.c.). A further repeated morphine-treated group received 20 min before naloxone, alprazolam (2.5 mg kg^{-1} s.c.).

2.5. Monoamine assay

Each sample of dialysate was injected directly onto a high-performance liquid chromatography system (HPLC) with electrochemical detection as described previously (Done et al., 1992). This system was comprised of an LKB Pharmacia 2150 solvent delivery system, Rainin column (4.6 × 150 mm, ODS C18, 5.0 μM particles) and a rheodyne 7125 injector fitted with a 25 μl loop and a BAS LC-4B electrochemical detector equipped with a single electrode analysis cell (MF1000). The glassy carbon electrode was held at +0.7 V with respect to a Ag/AgCl reference electrode. The mobile phase was set at a flow rate of 1.1 ml min^{-1} and contained: 0.1 M NaH_2PO_4, 2.0 mM sodium octane sulphonate, 0.5 mM EDTA and 12% (v/v) methanol with a final pH of 4.6 The limit of detection for noradrenaline was 20 fmol per sample at a 2 : 1 signal-to-noise ratio.

2.6. Locomotor activity

The effect of alprazolam in non-morphine-dependent rats on locomotor activity was investigated. Immediately after alprazolam or vehicle administration each rat was placed in a wooden box measuring 60 × 60 cm with 20 cm grids marked on the floor surface. The number of crossings the rat made in a 30 min period was recorded. The mean number of crossings for 8 rats from the alprazolam treatment group was compared with the vehicle group. The rats were also observed during this period for observable effects of alprazolam on overt behaviour.

2.7. Drugs

Morphine sulphate (Vestric, UK), naloxone hydrochloride (Sigma, UK) and alprazolam (Upjohn, Crawley, UK) were dissolved in normal pyrogen-free saline (0.9% w/v NaCl; Baxters, UK) and administered in a dose volume of 1 ml kg^{-1} either s.c. or i.p. as specified.

2.8. Data analysis

Data for the behavioural experiments are presented as either median and interquartile range for scored data or mean ± S.E.M. for interval level data. Statistical significance was determined using the Mann-Whitney *U*-test. The microdialysis data are presented as the absolute amounts in pmol of noradrenaline in each 20 μl sample. The effect of naloxone on noradrenaline output in rats treated repeatedly with morphine was assessed statistically using a two-way repeated measures analysis of variance procedure followed by post-hoc analysis with the Newman-Keuls multiple range test. Probability value of 0.05 or less was considered statistically significant.

3. Results

3.1. Effect of alprazolam on the physical behaviours expressed during naloxone-precipitated morphine withdrawal

In morphine-naive rats, alprazolam decreased spontaneous locomotor activity by 15% compared to vehicle controls. No other observable effect on overt behaviour was noted from the general behavioural screen.

In drug-naive rats, naloxone had no detectable effect upon behaviour (data not shown). In contrast, an injection of naloxone to rats treated repeatedly with morphine caused a clear-cut behavioural syndrome characterized by flat body posture, wet dog shakes, diarrhoea, hypothermia and weight loss (Table 1). Pretreatment with alprazolam (2.5 mg kg^{-1} s.c., 20 min prior to naloxone) significantly decreased mean body weight loss, cholinergic signs (i.e. lacrimation, urination, rhinorrhoea, salivation) of opiate withdrawal, squeal on touch, preventing forepaw tread/tremor, jumping and escape behaviours. Furthermore, alprazolam attenuated wet dog shakes, burrowing, teeth chattering, head weave/twitching and writhing. However, alprazolam had no effect against the opiate withdrawal-induced hypothermia.

3.2. Effect of alprazolam on naloxone-precipitated efflux of hippocampal noradrenaline in the anaesthetised rat

Fig. 1 illustrates that an injection of naloxone (1 mg kg^{-1} s.c.) 3 h after the last of 8 twice daily injections of morphine induced an immediate and sustained increase in noradrenaline efflux in hippocampal perfusates in morphine-dependent rats. The increased noradrenaline efflux was sustained beyond the observation period of 140 min. The maximal increase in noradrenaline was evident 40 min

Table 1
Effect of pretreatment with alprazolam on the naloxone-precipitated physical withdrawal syndrome in morphine-dependent rats

Behaviour	Control ($n = 12$)	Alprazolam ($n = 11$)
Wet dog shakes	15.0 (13.2–18.9)	5.0 (3.5–7.5) [b]
Burrowing	1.5 (0–2)	3.5 (2.5–5.0) [a]
Teeth chat	35.5 (30–45.5)	19.5 (19–28) [a]
Head weave	27.5 (24.5–42)	19.5 (16–21.5) [a]
Head twitch	5.0 (2.5–7.0)	2.0 (0–3.5)
Writhing	15.0 (8.5–20.0)	7.0 (5.5–10.5) [a]
Grooming	1.0 (0–2)	0
Forepaw tread	4.5 (2–8.5)	0 [b]
Forepaw tremor	8.5 (4.5–12.5)	0 [b]
Jumping	4.0 (0–6)	0 [b]
Escapes	10.0 (8–13.5)	0 [b]
Squeal on touch hostility	24.0 (20–24.5)	5.0 (2.5–8.5) [b]
Ptosis	18.0 (17.5–20)	6.0 (4–10.5) [b]
Salivation	18.0 (16–21)	8.0 (7.5–12.5) [b]
Rhinorrhoea	18.0 (16–21.5)	10.5 (8–12) [a]
Diarrhoea	20.0 (18.5–21.0)	8.0 (7.5–14.5) [b]
Urination	18.0 (15.5–21.0)	8.0 (6.5–13.5) [b]
Weight loss (g)	27.57 ± 5.6	12.32 ± 3.2 [b]
Reduction in body temperature (°C)	2.91 ± 0.9	1.99 ± 1.01

Alprazolam (2.5 mg kg^{-1} s.c.) was administered 20 min prior to naloxone in morphine-dependent rats. Each value is the median behavioural score (with interquartile range) accumulated over a 30 min (6 × 5 min observation periods). Temperature and weight measurements were monitored every 30 min upto 2 h post-naloxone administration. Each value represents mean ± S.E.M.) following naloxone (1 mg kg^{-1} i.p.) which was administered 3 h after the last dose of 8 daily injections of morphine to all groups. [a] $P < 0.05$, [b] $P < 0.01$.

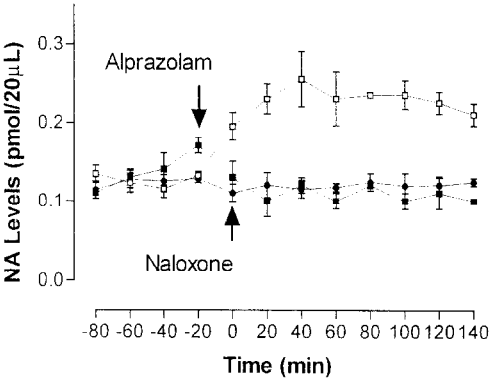

Fig. 1. Effect of naloxone on noradrenaline output in hippocampal microdialysates of morphine-naive and morphine-dependent rats. Naloxone (1 mg kg^{-1} i.p.) was administered in all groups. Data from three groups of rats are shown: morphine-naive (●); morphine-dependent rats with (■); and without (□) pretreatment with alprazolam (2.5 mg kg^{-1} s.c.). In all groups naloxone was injected 3 h after the last of 8 daily injections of morphine. Alprazolam was injected 20 min prior to naloxone. Results are means ± S.E.M. from groups of 6 rats. Two-way ANOVA with repeated measures showed a significant effect of treatment ($F(2,16) = 19.89$; $P < 0.001$).

post-naloxone, and represented levels of 210.4 ± 10.5% of noradrenaline output compared to basal. Morphine-dependent rats pretreated with alprazolam 20 min before naloxone prevented the naloxone-precipitated efflux of hippocampal noradrenaline (Fig. 1). The noradrenaline levels up to 140 min post-naloxone remained similar to the pre-naloxone levels.

4. Discussion

The present study has evaluated the interaction of acute alprazolam with naloxone-precipitated opiate withdrawal, to provide information on a possible mechanism through which benzodiazepines may reduce the severity of opiate withdrawal.

Following naloxone (1 mg kg^{-1} s.c.) administration to morphine-dependent rats a robust and reproducible physical withdrawal syndrome which comprised a range of overt and quantifiable behaviours, was observed as noted in Table 1.

When alprazolam was administered 20 min before naloxone, to a further chronic morphine group, the withdrawal syndrome produced by naloxone was markedly reduced in severity. Lal et al. (1971) consider social aggression (squeal on touch hostility) to be a reliable behavioural facet of acute opioid withdrawal. Thus a reduction in social aggression is an important manifestation of a diminished withdrawal syndrome. From the present study with alprazolam, squeal on touch hostility was virtually abolished and gives strong support for a decreased affective component to the withdrawal syndrome. These data further support the work of Gibert-Rahola et al. (1988) and Maldonado et al. (1991) who showed that various benzodiazepines modify the opiate withdrawal response in mice. Additionally, Tejwani et al. (1993) have shown that midazolam prevents naloxone-precipitated opiate withdrawal jumping.

The present microdialysis investigation contributes interesting and novel data on the effect of alprazolam on the naloxone-precipitated increase in hippocampal noradrenaline. The dramatic rise in noradrenaline following opiate antagonist-precipitated withdrawal was prevented following pretreatment with alprazolam. The magnitude of protection from pretreatment with alprazolam was greater than that with clonidine in a comparable study (Done et al., 1992).

It is clear from Fig. 1 that the basal level of noradrenaline during the alprazolam injection was higher than the other treatment groups. Two possible explanations for this may be: (1) due to some earlier fatalities the alprazolam treatment group had a lighter anaesthetic regimen, which may have resulted in higher levels of noradrenaline due to a decreased inhibition from the chloral hydrate on locus coeruleus activity; and (2) it is also possibile this group of rats had started to experience spontaneous opioid withdrawal which also resulted in elevated levels of noradrenaline.

Overall there is considerable support for the suggestion that clonidine attenuates the behavioural expression of morphine withdrawal by suppressing central noradrenergic neurotransmission at the presynaptic level (Redmond and Krystal, 1984; Done et al., 1992). It is unlikely that alprazolam would be acting through the same mechanism. In order to fully address the mechanism through which alprazolam attenuates the increase in noradrenaline in hippocampal perfusates following naloxone-precipitated withdrawal further research is required. The action of alprazolam on noradrenaline efflux in non-morphine- and morphine-dependent rats has to be investigated. Simpson and Weiss (1989) found that alprazolam and diazepam given peripherally were able to reduce both spontaneous and evoked activity of the locus coeruleus neurones, with evoked activity being more strongly attenuated. However, intracerebroventricular or intra-locus coeruleus administration attenutates the spontaneous activity of the locus coeruleus neurones but not the evoked activity. Furthermore, electrophysiological and immunocytochemical studies have identified an inhibitory γ-aminobutyric acid (GABA-ergic) input to the locus coeruleus from the prepositus hypoglossal nucleus (Ennis and Aston-Jones, 1989). It is conceivable, therefore, that the GABA potentiating effect of alprazolam inhibits the increased activity of the locus coeruleus during precipitated withdrawal via increased GABA-ergic activity from the prepositus hypoglossal nucleus.

In summary, this investigation has shown the ability of alprazolam to reduce the severity of the physical symptoms of opiate withdrawal in rats following acute treatment with this benzodiazepine. Additionally, this study has shown the capacity of alprazolam to prevent the recognized increased activity of the locus coeruleus during precipitated withdrawal, as reflected by increased noradrenaline levels in hippocampal dialysates following precipitated opioid withdrawal. One interpretation of the data suggests that alprazolam decreases the severity of the physical withdrawal syndrome by dampening down the hyperactivity of the locus coeruleus during withdrawal. However, Chieng and Christie (1995) have presented data which dispute the pivotal role of the locus coeruleus in morphine withdrawal and thus the possibility cannot be excluded that the decreased severity of the withdrawal syndrome following alprazolam pretreatment may be due to the anxiolytic properties of alprazolam.

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