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Naloxone blocks the antianxiety but not the motor effects of benzodiazepines and pentobarbital: experimental studies and literature review

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Abstract The role of opioid systems in the anticonflict effect of chlordiazepoxide, diazepam and pentobarbital was evaluated with a modified Vogel procedure. First, morphine, ineffective by itself, was combined with subeffective or marginally effective doses of the benzodiazepines in order to detect possible potentiation. However, the combined treatment reduced licking in the Vogel procedure as well as in a licking test where no shock was administered. Several doses of the benzodiazepines and pentobarbital were then administered in combination with several doses of the opiate antagonist naloxone. A dose-dependent inhibition of anticonflict effect was obtained. In an additional experiment, it was shown that naloxone blocked the effects of diazepam in the elevated plus-maze procedure. Motor deficiencies, as evaluated with a rotarod test, produced by the benzodiazepines and pentobarbital could not be antagonized by naloxone. It is concluded that opioids are important for the anticonflict but not for the motor effects of these drugs. An analysis of published studies concerning the interaction of opioids and benzodiazepines in several procedures supposed to reflect anxiolytic effects shows that the inhibition obtained with naloxone is reliable and not procedure specific. The mechanisms by which opiate antagonists produce this inhibition of anticonflict activity are not known. It is tentatively suggested that opioid activation associated with stress may be a necessary component of anxiolysis.

Key words Benzodiazepines • Pentobarbital • Opioids • Vogel procedure • Anxiety • Motor deficiencies

Introduction

Despite the spectacular progress in the molecular biology and pharmacology of benzodiazepine receptors (Doble and Martin 1992; Giusti and Arban 1993), much behavioral data concerning the actions of benzodiazepines remains unexplained. For example, it has been reported that several effects of benzodiazepines are blocked by opiate antagonists (see Millan and Duka 1981 for review). Anticonflict effects of diazepam and chlordiazepoxide are antagonized by naloxone in the Geller-Seifter or Vogel procedures (Billingsley and Kubena 1978; Soubrie et al. 1980; Duka et al. 1981; Koob et al. 1980). Stimulatory actions of diazepam on food intake in a stressful environment have also been blocked by naloxone (Soubrie et al. 1980). Furthermore, the enhanced intake of food and water in familiar situations produced by several benzodiazepines can be blocked by naloxone, independently of whether sated or deprived subjects are used (reviewed in Cooper 1983). Finally, benzodiazepine-facilitated intracranial self-stimulation (Lorens and Sainati 1978) and conditioned place preference produced by diazepam (Spiraki et al. 1985) are blocked by naloxone.

Some biochemical data appear to support the hypothesis that endogenous opioids may be related to benzodiazepine actions. Benzodiazepines have been found to modulate enkephalin release (Duka et al. 1979; Wiirster et al. 1980; Harsin et al. 1982) and this effect is blocked by naloxone (Duka et al. 1980). Moreover, systemic administration of morphine and intracerebroventricular infusion of β-endorphin enhance benzodiazepine binding (Lopez et al. 1990; Gomar et al. 1993b) while naloxone reduces it (Gomar et al. 1993a). The mechanisms behind these effects are not clear.
However, recent data have challenged the hypothesis that opiate antagonists efficiently block the effects of benzodiazepines. Cannizaro et al. (1987) and Tripp and McNaughton (1991) found naloxone unable to inhibit the effects of chloralhydrate in modified Geller-Seifter procedures, and the latter authors obtained similar results with a successive discrimination procedure (Tripp and McNaughton 1987). This coincides with an earlier report (Herling 1983). Furthermore, naloxone has been reported to reduce intake of food and water by itself in both sated and deprived animals (reviewed in Levine et al. 1985; Reid 1985). Similarly, naloxone reduces intracranial self-stimulation in a dose dependent way in the absence of other drug treatment (Schaefer 1988). This makes it difficult to interpret the proposed antagonism of benzodiazepine-enhanced eating, drinking and self stimulation. There is, in addition, a report in which naloxone was found to be unable to reduce diazepam-induced hyperphagia in a stressful environment but effectively antagonized the hyperphagia observed in a familiar environment (Britton et al. 1981). The authors proposed that the primary effect of naloxone is on consummatory behavior rather than on anticonflict actions.

Most of the studies mentioned above employed only one dose of the benzodiazepine and one dose of naloxone. Occasionally, two doses of each were used. It seems, then, that the contradictory findings may be partly explained by discrepancies in doses. One purpose of the present studies was, therefore, to evaluate the ability of several doses of naloxone to block the anticonflict effects of several doses of benzodiazepines in the Vogel procedure. Furthermore, the interaction between diazepam and naloxone was analyzed in the elevated plus-maze (Pellow et al. 1985). It was considered important to use a procedure where no painful stimulation is employed, because of the complex interplay between benzodiazepines and opiates in the mediation of analgesia (Maier 1990; Harris and Westbrook 1994).

The supposed interaction between benzodiazepines and opiates does not seem to apply to other drugs acting at the GABA/benzodiazepine/barbiturate/steroid receptor. In studies where naloxone blocked the effects of benzodiazepines on conflict behavior and on food and water intake, the effects of barbiturates were not blocked (Billingsley and Kubena 1978; Cooper and McGivern 1983; Naruse et al. 1989). The second purpose of the present studies was to determine whether naloxone could block the anticonflict effect of pentobarbital in the Vogel procedure.

If the opiate antagonist naloxone blocks the anticonflict effects, it could be supposed that opiates should be anxiolytic. Experimental evidence for this supposition is weak, however (reviewed by Pollard and Howard 1990). Nevertheless, clinical studies have shown a substantial prevalence of benzodiazepine use among opiate addicts (Brown and Chaitkin 1981; Darke et al. 1993) and methadone maintenance patients report that diazepam enhances the effect of methadone (Kleber and Gold 1978; Stitzer et al. 1981). Even if opiates do not display anxiolytic properties by themselves, it is possible that they may potentiate or be potentiated by benzodiazepines. This was also evaluated in the present experiments.

Most benzodiazepines and barbiturates produce motor deficiencies in doses somewhat larger than those required for anticonflict effects (Ágmo and Fernandez 1991; Ágmo et al. 1991). The last purpose of the present studies was therefore to evaluate the capacity of naloxone to block the motor incoordination produced by benzodiazepines and pentobarbital.

**Materials and methods**

**Subjects**

Male Wistar rats (250–350 g) from a local colony were housed under a natural light/dark cycle at an ambient temperature of 22 ± 1°C. They were kept five per cage, and given commercial rat pellets ad lib. Tap water was freely available until 24 h before conflict experiments. For the plus-maze experiments, male Wistar rats were purchased from Janvier, Le Genest Saint Isle, France. These rats were maintained under a 12/12-h light/dark cycle, two per cage.

**Apparatus and procedure**

The lickometer has been described in detail elsewhere (Ágmo et al. 1991). Briefly, an optical lickometer was mounted in a standard operant cage. Shocks (square pulses of 5 ms duration with a frequency of 100 Hz) were generated by a Grass S48 stimulator connected to a Grass constant current unit adjusted to 0.25 mA. At the test session, shock was applied between the drinking spout and the grid flood for 5 s after every 20 shock free licks. The latency to lick, the total number of licks and the number of licks during shock were registered by a BRS/LVE electromechanical equipment. The lickometer was located in a sound attenuating cage in a room adjacent to the control equipment.

After 24 h of water deprivation, the rats were allowed to drink in the lickometer apparatus for 5 min in the absence of shock. They were then returned to the animal quarters and allowed to drink for another 20 min. Any subject that made fewer than 200 licks in the lickometer was eliminated. This was to ensure that only rats that actually licked were included in the test. A rat that did not lick, or made fewer than 20 licks, would receive no shock, and there would be no conflict. The following day, at the same hour, the 5-min test was made. Here, the drugs were administered before licking and shock was applied as described above. It has previously been shown that this version of the Vogel procedure (Vogel et al. 1971) is not sensitive to variations in motivation to drink, motor effects of drugs or analgesia. Administration of analgesics or increasing the water deprivation from 24 to 48 h do not modify licking at the test. Moreover, benzodiazepines in doses that impair motor execution require for anticonflict effects (Agmo and Fernandez 1991; Agmo et al. 1991).