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BRIEF COMMUNICATION

Pentobarbital-Induced Hyperactivity in Mice: Negligible Role of Opioid Mechanisms

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VETULANI, J., F. PAVONE, M. BATTAGLIA AND M. SANSONE. *Pentobarbital-induced hyperactivity in mice: Negligible role of opioid mechanisms*. PHARMACOL BIOCHEM BEHAV 33(4) 927–929, 1989.—Subhypnotic doses (10 and 20 mg/kg) of pentobarbital significantly elevated locomotor activity measured for 30 min in CD-1 mice. The hyperactivity was also observed in mice recovering from pentobarbital-induced (50 mg/kg) sleep (measurements starting 15 min after recovery of righting reflex). Naloxone in doses up to 4 mg/kg did not affect significantly the pentobarbital-induced hyperactivity in any experiment; a dose of 8 mg/kg only partially attenuated the hyperactivity induced by a dose of 20 mg/kg of pentobarbital, but did not affect significantly either the stimulatory effect of a low subhypnotic dose (10 mg/kg) or the posthypnotic hyperactivity. This suggests a negligible involvement of opioid mechanisms in the hyperactivity induced by pentobarbital.

Naloxone Pentobarbital Locomotor activity Mice

BARBITURATES and benzodiazepines exert many of their actions by binding to specific domains of the GABA receptor-chloride channel complex (8), and because of that a part of their behavioral profile is similar [see (6)]. Although generally regarded as sedatives, under some conditions, benzodiazepines [see (13)] and barbiturates (16) may stimulate locomotor activity.

The hyperactivity and overexcitement induced by barbiturates is particularly interesting as it is regarded as one of the unwanted but not uncommon side-effects of the drugs given as hypnotics. Moreover, such an excitement is also observed as an after-effect of barbiturate anesthesia (6).

Although the involvement of neurotransmitter systems downstream of the primary receptor event in barbiturate- and benzodiazepine-induced locomotor stimulation is not completely clear as yet, it was postulated that catecholaminergic mechanisms are involved in this phenomenon (9, 14, 15). In addition, the participation of opioid mechanisms in the hyperactivity induced by chlordiazepoxide has recently been demonstrated (9). In the present paper we investigated if the opioid mechanisms are also involved in pentobarbital-induced hyperactivity caused by high but not hypnotic doses of the barbiturate (subhypnotic doses) and the hyperactivity appearing shortly after the recovery of righting reflex loss resulting from a treatment with a hypnotic pentobarbital dose (posthypnotic hyperactivity). Our results indicate that both hyperactivity induced by subhypnotic doses and particularly the posthypnotic hyperactivity are fairly resistant to the action of naloxone, suggesting relatively small, if any, participation of opioid mechanisms in this action of barbiturates.

METHOD

The subjects were naive male mice (28–33 g) of the randomly bred CD-1 strain (Charles River, Italy). Upon their arrival in the laboratory (7–10 days before the experiment), the mice were housed in standard transparent plastic cages (8 per cage), under standard laboratory conditions (free access to food and water, ambient temperature of 22°C, 12-hr light/dark cycle, light on at 7 a.m.).

The locomotor activity was measured in an apparatus consisting of 8 toggle-floor boxes, each divided into two 20 × 10 cm compartments connected with a 3 × 3 cm opening. For each mouse, the number of crossings from one compartment to the

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other was automatically recorded by means of a microswitch connected to the tilting floor of the box. The apparatus was located in a sound-insulated cubicle. Mice were subjected, only once, to a 30-min activity test, between 9 a.m. and 3 p.m.

In the first experiment, mice were taken from their home cages for injection and returned to it (8 per cage) till testing. Subhypnotic doses of pentobarbital sodium (Clin-Midi, France; 0, 10 or 20 mg/kg) were given in combination with various doses of naloxone hydrochloride (Endo; 0, 1, 2, 3, 4 or 8 mg/kg) to groups of 16 mice. Combinations of drugs were given as mixed solutions, in a single injection; the controls (dose 0 of both drugs) received saline solution (0.9% NaCl). All injections were made intraperitoneally in a volume of 10 ml/kg.

In the second experiment, mice received a sleep-inducing dose (50 mg/kg) of pentobarbital sodium. The mice used for this experiment and the appropriate controls were placed in single $30 \times 12 \times 12$ cm transparent plastic cages, where they remained till the test for locomotor activity. Forty-eight hr after separation they received injections of saline or pentobarbital. The pentobarbital-treated mice in which sleeping time (loss of righting reflex) was shorter than 30 min or longer than 2 hr were rejected. The selected mice were divided into groups of 8. The animals were injected with naloxone hydrochloride (0, 1, 2, 4, or 8 mg/kg) soon after recovery of righting reflex; the saline-treated controls received the second injection 60–80 min after the first one (the variability of between-injection interval intended to account for the difference in the individual sleeping times of pentobarbital-treated subjects). Fifteen minutes later mice were placed in activity cages.

The results were analyzed by nonparametric statistical methods. Median number of crossings and interquartile range (Q1–Q3) were calculated for each experimental group and significance of the differences between groups were evaluated by means of the Kruskal-Wallis one-way ANOVA followed, when appropriate, by the Mann-Whitney U-test; for assessment of correlation the Pearson's r coefficient was used (11).

RESULTS

Table 1 shows the locomotor effects produced by subhypnotic doses of pentobarbital, given alone or in combination with naloxone. Pentobarbital alone produced hyperactivity when given in a dose of 10 mg/kg (an increase in the median score by about 30%); the effect of a dose of 20 mg/kg was much stronger (increase by about 90%). Naloxone alone (1–8 mg/kg) did not produce any significant effect, and in most cases did not affect the pentobarbital hyperactivity. The highest dose of naloxone (8 mg/kg) did not affect at all the hyperactivity produced by the low dose of pentobarbital, but significantly attenuated (depression of the median by 30%) the hyperactivity induced by 20 mg/kg of pentobarbital. Even at this dose, however, naloxone did not abolish completely the stimulatory effect of the barbiturate.

The dose of 50 mg/kg of pentobarbital produced sleep lasting between 39 and 114 min (73 min on average), which was followed by a period of locomotor activity significantly higher than in the saline-treated controls (the median higher by about 120%) (Fig. 1). The posthypnotic activity was resistant to the action of naloxone administered soon after recovery of righting reflex, as revealed by Kruskal-Wallis one-way ANOVA for the activity crossings exhibited by the five groups, receiving pentobarbital and naloxone (0, 1, 2, 4, 8 mg/kg), which indicated no significant between-group difference.

No correlation between the duration of pentobarbital sleep and the posthypnotic activity was found in any group.

DISCUSSION

Sedative drugs such as benzodiazepines and barbiturates may, under certain circumstances, produce excitement in man. The animal equivalent of this effect of sedative-hypnotics seems to be an excessive locomotor stimulation. The locomotor stimulatory effect of subhypnotic doses of barbiturates in mice has been long since known [see (16)], and we presently report that it appears also as an aftereffect following pentobarbital-induced general anesthesia. As excitatory effects of benzodiazepines and barbiturates are clinically undesirable, the mechanisms underlying them are of obvious interest.

It is generally thought that the excitatory effects of sedative-hypnotics result from the inhibition of inhibitory pathways. Thus, for pentobarbital, it was proposed that it produces an inhibition of central cholinergic pathways, which results in a preponderance of dopaminergic system (15). As we have previously reported (9) that the hyperactivity induced by chlordiazepoxide is effectively inhibited by naloxone, the participation of opioid mechanisms in the benzodiazepine-induced hyperactivity is also suggested.

Opioid mechanisms were known to play a role also in some actions of barbiturates, such as anesthesia and toxicity (4). Because of that and because of several analogies between the effects of barbiturates and benzodiazepines, we expected that opioid antagonist would also attenuate pentobarbital-induced hyperactivity, but we found that the pentobarbital-induced hyperactivity is naloxone-resistant; it is slightly affected only by a high dose of naloxone, which cannot be regarded as opioid-specific.

This discrepancy between the results obtained with chlordiazepoxide and pentobarbital was unexpected, as the pharmacological profile of both barbiturates and benzodiazepines is similar, and both classes of compounds act on particular domains of the GABA receptor-chloride channel complex, and both potentiate the effects of GABA by facilitating the influx of chloride into the neuron (5, 8, 17, 18).

TABLE 1

EFFECT OF NALOXONE AND PENTOBARBITAL ON LOCOMOTOR ACTIVITY IN MICE

Naloxone (mg/kg)	Pentobarbital (mg/kg) 0	10	20	H (2)
0	74 (61–92)	96 (79–114)	140[a] (113–166)	23.41†
1	67 (53–86)	86[a] (75–109)	113[a] (92–141)	13.23†
2	72 (53–84)	96[a] (71–121)	123[a] (105–158)	15.92†
4	68 (52–80)	101[a] (73–117)	117[a] (108–122)	21.57†
8	71 (59–92)	104[a] (77–120)	98[b,c] (75–124)	6.61*
H (4)	1.81	0.53	9.73*	

Median activity crossings, with interquartile ranges (Q1–Q3; in parentheses) in groups of 16 animals receiving naloxone hydrochloride and sodium pentobarbital, alone or in combination, 15 min before testing.

Asterisks denote significance (*$p<0.05$; †$p<0.001$) in the Kruskal-Wallis one-way ANOVA (values of H; *df* in parentheses) for each dose of naloxone or pentobarbital.

Significances ($p<0.05$) in the Mann-Whitney U-test: [a]Pentobarbital alone vs. saline (dose 0) and drug combinations vs. NX alone, at the corresponding doses; [b]Drug combinations vs. pentobarbital alone, at the corresponding doses; [c]Borderline significance ($p=0.052$) of difference between this group and the group receiving naloxone 8 mg/kg without pentobarbital.

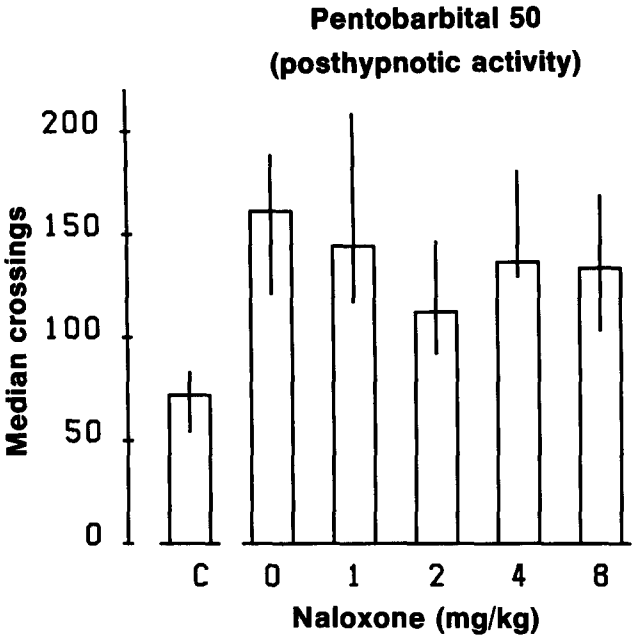

FIG. 1. Locomotor activity (median crossings), measured during 30 min, in groups of eight mice subjected to the activity test 15 after awakening from pentobarbital anesthesia (50 mg/kg/IP). Naloxone was injected soon after the recovery of the righting reflex. Vertical lines indicate interquartile ranges (Q1–Q3). The activity produced by pentobarbital (0 mg/kg naloxone) was significantly higher ($p<0.02$; Mann-Whitney U-test) than in saline-treated control group (C). No significant difference ($p=0.60$; Kruskall-Wallis test) between groups receiving various doses of naloxone after barbiturate sleep was observed.

Nevertheless, some differences in the action of benzodiazepines and barbiturates exist both on the molecular and pharmacological level. Thus, benzodiazepines increase the chloride influx by increasing the frequency of chloride channel opening, while barbiturates prolong the channel opening time (12). Moreover, while benzodiazepines seem to act on the chloride channel only by potentiating the action of GABA (7) and seem only to enhance the GABA-ergic inhibition at synapses that demonstrate tonic GABA-ergic transmission (2,5), barbiturates may also act on the chloride channel by other mechanisms (2). In high doses, barbiturates may act directly on the ionophore, increasing the chloride influx even in the absence of GABA (1,10) and hence may enhance inhibition at synapses that are already maximally inhibited by tonic GABA-ergic input (18). This explains why barbiturates are much more toxic than benzodiazepines.

The present results suggest a different involvement of opioid mechanisms in the locomotor stimulatory action of benzodiazepines and barbiturates. A similar difference in the action of naloxone on another effect of benzodiazepines and barbiturates, namely hyperdypsia, was reported by Cooper (3). Thus, despite several similarities between benzodiazepines and barbiturates, some of their stimulatory effects are distinguishable by various interactions with opiate antagonists.

The involvement of opioid mechanisms in some actions of benzodiazepines but not barbiturates suggests that the opioid mechanisms involved in the modification of the action of benzodiazepines do not operate downstream of the GABA receptor-chloride channel complex [as we suggested previously (9)], but that they may belong to those mechanisms that regulate responsiveness to GABA at the membrane level (5). If the locomotor hyperactivity produced by both classes of drugs is mediated by their interaction with the GABA receptor, the opioid mechanisms modulating this response should operate on the level between the benzodiazepine receptor and chloride channel, while the coupling of benzodiazepine binding site with the chloride channel, involved in the excitatory response, apparently bypasses a step controlled by opioid mechanisms.

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