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Similar effects of nifedipine and hydralazine on anaesthesia and hypermotility induced by pentobarbitone in mice

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Abstract—Nifedipine, a dihydropyridine calcium channel blocker, and hydralazine, a non-calcium channel antagonist vasodilatator, enhanced pentobarbitone-induced sleeping time and reversed locomotor hyperactivity induced by a subhypnotic dose of the barbiturate in mice. The similarity of the behavioural effects, exerted by nifedipine and hydralazine, suggest that haemodynamic factors may play an important role in the interaction of calcium channel antagonists with barbiturates.

Calcium plays an important role in different neuronal functions (Miller 1987) and mediates some central drug effects such as sedative-hypnotic actions (Carlen & Wu 1988). Consequently, calcium channel blockers may interfere with some neuronal activities and with the action of centrally acting drugs (Turkkan 1988). Thus, dihydropyridine calcium channel antagonists, like nimodipine (Hoffmeister et al 1982) and nitrendipine (Dolin & Little 1986), as well as structurally different compounds, like verapamil and flunarizine (Dolin & Little 1986), potentiate barbiturate anaesthesia. However, the doubt has been raised that changes in cerebral blood flow, produced by calcium antagonists, may contribute to enhance the anaesthetic potency of the barbiturates (Dolin & Little 1986).

In the present study, nifedipine, a dihydropyridine calcium blocker, and hydralazine, a non-calcium channel antagonist vasodilator (Rudd & Blaschke 1985), were compared for their interaction with pentobarbitone. The barbiturate was tested for its anaesthetic properties and for its ability to induce locomotor hyperactivity in mice, when given at subhypnotic doses (Harris et al 1966; Watzman et al 1968; Middaugh et al 1981; Sansone et al 1989; Vetulani et al 1989). Biochemical experiments were also carried out to investigate whether nifedipine affects pentobarbitone concentration in the mouse brain.

Materials and methods

Animals and drugs. Behavioural experiments were carried out on naive male mice, 30–35 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, light on from 0700 to 1900 h). The experiments were carried out between 0900 and 1600 h. Biochemical experiments were carried out, in Krakow, on male Albino Swiss mice.

Nifedipine (Sigma), dissolved in 50% polyethylene glycol, mol. wt 400 (PEG, Sigma), and hydralazine hydrochloride (Sigma), dissolved in distilled water, were injected intraperitoneally in a volume of 4 mL kg^{-1}. Pentobarbitone sodium (Clin-Midi, France) was injected intraperitoneally in a volume of 10 mL kg^{-1}.

Anaesthesia. Mice were placed in single 30 × 12 × 12 cm transparent plastic cages and received nifedipine or hydralazine hydrochloride, at the doses of 0 (PEG and 0·9% NaCl (saline), respectively), 1, 2·5, 5 or 10 mg kg^{-1}. Thirty min after nifedipine or hydralazine, the animals received a sleep-inducing dose (50 mg kg^{-1}) of pentobarbitone sodium. Each experimental group consisted of 10 animals. Sleeping time was measured as the time interval between loss and recovery of the righting reflex. A mouse was considered awake upon righting three times in 30 s.

Locomotor activity. Thirty min after treatment with nifedipine or hydralazine hydrochloride (0, 1, 2·5, 5 or 10 mg kg^{-1}), mice received saline or a subhypnotic dose of pentobarbitone (20 mg kg^{-1}), chosen on the basis of previous experience (Sansone et al 1989; Vetulani et al 1989). Mice were subjected to the activity test, starting 15 min after the second injection. Each experimental group consisted of 8 animals. The locomotor activity was measured, over 30 min, in an apparatus consisting of 8 toggle-floor boxes, each divided into two 20 × 10 cm compartments connected by a 3 × 3 cm opening. For each mouse, the number of crossings from one compartment to the 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.

Biochemistry. Mice treated with PEG or nifedipine (10 mg kg^{-1}) received, 30 min later, pentobarbitone in a dose of 20 or 50 mg kg^{-1} and were killed by decapitation 15 min after barbiturate injection. The brains were rapidly removed. Single brains were used for determination of pentobarbitone in the mice treated with the high dose; for the assay of the compound in brains of mice receiving 20 mg kg^{-1} of pentobarbitone two brains were combined.

The brains were homogenized in 3 mL of 0·01 M HCl, the homogenate was extracted with 10 mL chloroform, and the chloroform extract was re-extracted with 2·5 mL of 0·01 M NaOH. The extracts were measured for pentobarbitone absorbance at wavelength range 200–300 nm, using a Beckman DU-65 spectrophotometer. The peak at 244 nm was used for quantification. The sensitivity limit was 3 μg of pentobarbitone in a single brain sample.

Results

Anaesthesia. Both nifedipine and hydralazine dose-dependently prolonged sleeping time induced by 50 mg kg^{-1} pentobarbitone (Fig. 1). Significant differences between groups resulted from overall analyses of variance, carried out separately for the two drug treatments ($F(4,45) = 14·59$ and 16·71, respectively, $P < 0·001$). Individual between-group comparisons (Duncan's test) indicated a significant effect of nifedipine at the doses of 5 and 10 mg kg^{-1}, while hydralazine prolonged sleeping time at all tested doses (1–10 mg kg^{-1}).

Locomotor activity. At the dose of 20 mg kg^{-1}, pentobarbitone did not produce loss of the righting reflex in PEG or saline pretreated animals. Conversely, several mice (3–5 out of 8) pretreated with 5 and 10 mg kg^{-1} of nifedipine or hydralazine showed loss of the righting reflex 15 min after the administration of the barbiturate, at the beginning of the activity test. However, these mice recovered the righting reflex and started to move

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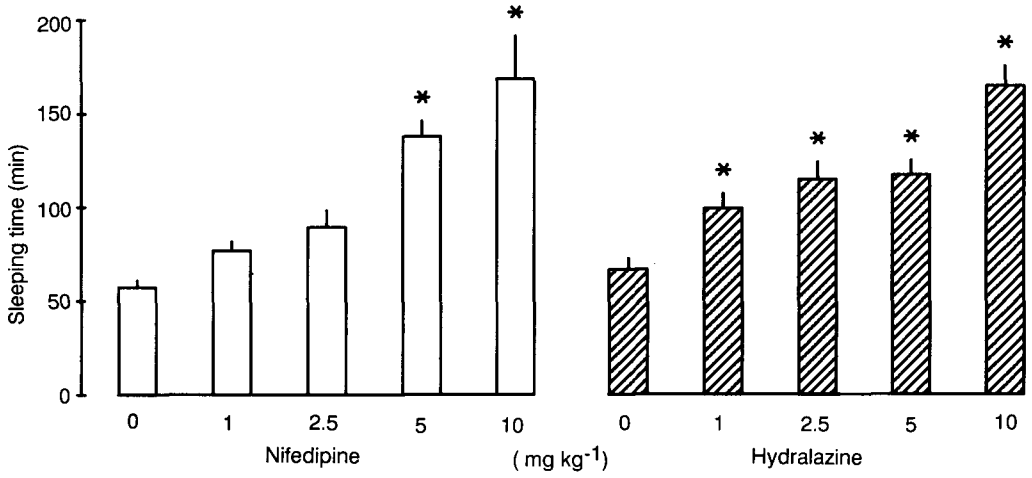

FIG. 1. Sleeping time induced by pentobarbitone sodium (50 mg kg^{-1}, i.p.), given 30 min after nifedipine or hydralazine hydrochloride. Columns represent mean values, in groups of 10 mice; vertical lines indicate s.e.m. $*P < 0.05$ compared with corresponding vehicle control.

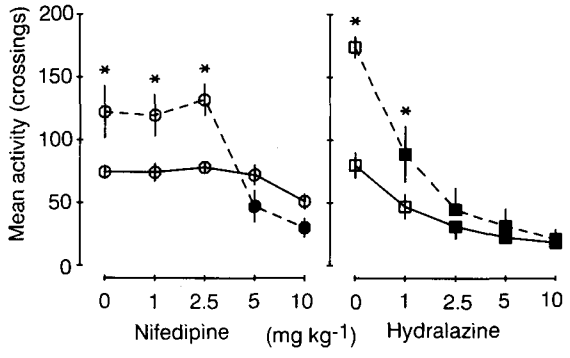

FIG. 2. Locomotor activity measured, over 30 min, in mice receiving saline (——) or pentobarbitone sodium (20 mg kg^{-1}) (- - - -), 30 min after nifedipine or hydralazine hydrochloride. The activity test was started 15 min after the second injection. Mean activity crossings, in groups of 8 mice; vertical lines indicate s.e.m. $*P < 0.05$ compared with corresponding controls. Closed symbols indicate a significant difference ($P < 0.05$) vs dose 0 of nifedipine and hydralazine, given alone or in combination with pentobarbitone.

during the 30-min test, except two animals pretreated with hydralazine (one at 5, the other at 10 mg kg^{-1}).

The mean activity crossings, exhibited by the mice in the 30-min test, are reported in Fig. 2 for all the experimental groups. A two-way analysis of variance, concerning pentobarbitone and nifedipine, showed significant main effects of the barbiturate ($F(1,70) = 8.17$, $P < 0.01$) and of the calcium antagonist ($F(4,70) = 12.76$, $P < 0.001$) and a significant drug interaction ($F(4,70) = 6.24$, $P < 0.001$), allowing a further analysis with Duncan's test (Fig. 2). Pentobarbitone stimulated locomotor activity in PEG-pretreated mice; nifedipine, given alone, produced a slight, non-significant depressant effect, only at a dose of 10 mg kg^{-1}, but reversed pentobarbitone-induced hyperactivity at doses of 5 and 10 mg kg^{-1}. Stronger depressant effects were produced by hydralazine. A two-way analysis of variance gave significant pentobarbitone ($F(1,70) = 18.42$, $P < 0.001$) and hydralazine ($F(4,70) = 26.90$, $P < 0.001$) main effects and a significant drug interaction ($F(4,70) = 4.98$, $P < 0.001$). Hydralazine significantly reduced locomotor activity, when given alone at doses of 2.5 to 10 mg kg^{-1}, and reversed pentobarbitone-induced hyperactivity even at 1 mg kg^{-1} (Duncan's test; Fig. 2).

Table 1. Effect of nifedipine on pentobarbitone concentration in the mouse brain.

Pentobarbitone (mg kg^{-1})	Pretreatment	
	PEG	Nifedipine
20	7.34 ± 0.94 (9)	11.25 ± 1.57* (7)
50	26.59 ± 2.43 (10)	24.45 ± 2.19 (10)

Brain pentobarbitone concentration ($\mu g\ g^{-1}$), measured 15 min after intraperitoneal administration of the barbiturate, in mice pretreated (30 min) with PEG or 10 mg kg^{-1} nifedipine. Mean values ± s.e.m.; number of brain samples in parentheses. $*P < 0.05$ (Student's t-test) between nifedipine and PEG.

Biochemistry. Nifedipine (10 mg kg^{-1}) significantly increased, by 53%, pentobarbitone concentration in the brain of mice receiving 20 mg kg^{-1} of the hypnotic, but did not affect barbiturate concentration after a dose of 50 mg kg^{-1} (Table 1).

Discussion

The present results demonstrate that both nifedipine and, to a greater extent, hydralazine, reversed locomotor hyperactivity induced by a subhypnotic dose of pentobarbitone and prolonged pentobarbitone-induced sleeping time.

Hydralazine reversed pentobarbitone hyperactivity even at the doses of 1 and 2.5 mg kg^{-1}, which, however, depressed locomotion also when given alone; conversely, at these dosage levels, nifedipine had no effect either alone or in combination with pentobarbitone. Reversal of pentobarbitone-induced hypermotility by the highest doses (5 and 10 mg kg^{-1}) of nifedipine can be explained on the basis of the biochemical findings, showing enhancement by the calcium antagonist (10 mg kg^{-1}) of pentobarbitone concentration in the brain of mice receiving 20 mg kg^{-1} of the hypnotic. It seems likely that, when the two drugs are given in combination at these doses, nifedipine produces vasodilatation of cerebral vessels and facilitates penetration of pentobarbitone into the brain. The same mechanism could explain the similar behavioural effects produced by hydralazine, when combined with the subhypnotic dose of pentobarbitone. At present, we have no biochemical findings to

support this hypothesis, since we have no data concerning concentration of the barbiturate in the brain of mice pre-treated with hydralazine. However, it must also be noted that pentobarbitone, at a dose of 20 mg kg^{-1}—which stimulated locomotor activity in control animals—produced a short-lasting loss of the righting reflex in about 50% of mice pre-treated with the highest doses of either nifedipine or hydralazine. This further similarity, in the interaction of nifedipine and hydralazine with pentobarbitone, validates the hypothesis that hydralazine, like nifedipine, may increase brain concentrations of pentobarbitone, following the administration of a subhypnotic dose of the barbiturate.

The prolongation of pentobarbitone-induced sleeping time by nifedipine is in agreement with previous findings showing potentiation of barbiturate general anaesthesia by calcium channel antagonists (Hoffmeister et al 1982; Dolin & Little 1986). This effect does not seem attributable to an interference of calcium antagonists with barbiturate pharmacokinetics. The present results demonstrate that, in mice receiving an anaesthetic dose of pentobarbitone, nifedipine did not change brain concentration of the barbiturate, in agreement with previous findings showing that nifedipine, another dihydropyridine calcium channel blocker, did not affect barbiturate plasma levels (Dolin & Little 1986); however, a slower elimination of the barbiturate from brain tissues cannot be excluded. Further experiments are required to clarify this point, by measuring brain concentration of pentobarbitone at varying times after treatment. According to Dolin & Little (1986) the enhancement of the barbiturate anaesthetic potency by calcium channel blockers can be due to a potentiation of the decreasing action on synaptic calcium entry, exerted by general anaesthetics. However, on the basis of the present results, it cannot be excluded that haemodynamic effects of calcium antagonists may contribute to prolonged barbiturate anaesthesia, since pentobarbitone-induced sleeping time was also prolonged by hydralazine. Previous studies demonstrated that calcium channel blocking agents exert some behavioural effects independently from their hypotensive actions (Bolger et al 1986; Turkkan & Hienz 1990). However, hypotensive effects could play a greater role when calcium antagonists are combined with barbiturates, which can themselves decrease arterial blood pressure and cerebral blood flow, at anaesthetic doses (Marshall & Wollman 1985). An abrupt fall in blood pressure, below the lower limit of the cerebral blood flow autoregulation, may produce cerebral hypoperfusion and impairment of central nervous functions (Turkkan 1988). Such circulatory interference, if produced by a combination of high doses of antihypertensive drugs with an anaesthetic dose of barbiturate, could increase brain sensitivity to depressant and toxic effects of the barbiturate. In this respect, it seems important to note that when pentobarbitone (50 mg kg^{-1}) was given after a higher dose (20 mg kg^{-1}) of nifedipine or hydralazine (data not shown), not only was the loss of righting reflex further prolonged, but 2 out of 8 mice died.

In conclusion, even if changes in calcium transport at synaptic levels seem to play a determinant role in the potentiation of barbiturate anaesthesia by calcium channel blockers, the present results suggest that the role of haemodynamic factors should not be disregarded. The present findings demonstrate that nifedipine, a calcium channel blocker and hydralazine, a non-calcium channel antagonist vasodilatator, interact similarly with the behavioural effects of pentobarbitone.

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