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BRIEF COMMUNICATION

Pregnenolone Sulfate Antagonizes Barbiturate-Induced Hypnosis

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MAJEWSKA, M. D., M.-T. BLUET-PAJOT, P. ROBEL AND E.-E. BAULIEU. *Pregnenolone sulfate antagonizes barbiturate-induced hypnosis.* PHARMACOL BIOCHEM BEHAV **33**(3) 701–703, 1989.—The potential influence of the neurosteroid pregnenolone sulfate (PrS) on barbiturate-induced hypnosis was tested in rats. PrS, when injected intracerebroventricularly or intraperitoneally, significantly shortened the sleep-time produced by pentobarbital. The results suggest an important physiological and pharmacological role for PrS in the regulation of CNS excitability.

Neurosteroids Pregnenolone sulfate Barbiturate hypnosis

THE central nervous system (CNS) has the capacity for de novo biosynthesis of several neurosteroids, which show altered regional concentrations associated with physiological states such as stress, sexual activities or diurnal cycles (1). We have shown recently that the neurosteroid, pregnenolone sulfate (PrS), behaves in vitro as an antagonist of the Cl^- channel that is coupled to the receptor for the inhibitory neurotransmitter, γ-aminobutyric acid (GABA) (8,9). The observation that PrS counteracts the neuronal electrophysiological responses to pentobarbital (PB), which opens the Cl^- channels associated with $GABA_A$ receptors (15), suggested that PrS might also antagonize in vivo the pharmacological effects of anesthetic barbiturates. We tested this hypothesis and report now that PrS injected either intracerebroventricularly (ICV) or intraperitoneally (IP) into rats significantly shortens pentobarbital-induced hypnosis.

METHOD

Experimental animals were maintained in a temperature- and humidity-controlled vivarium on 0600–1800 hr lighting schedule. Two strains of rats were used due to animal availability, because the experiments were performed in two geographical places (the USA and France). Male Sprague-Dawley rats (350 g) were used for experiments in which PrS was injected ICV, and Fischer-344 rats (250 g) were used for experiments in which PrS was injected IP. In both cases rats were administered an IP injection of sodium pentobarbital (PB; 45 mg/kg) dissolved in saline.

Experiments were started in the early afternoon and drugs were given in a random order. The ICV steroid injections were administered to pentobarbital-injected rats that were placed in a stereotaxic instrument as soon as they were anesthetized. The incisor bar was positioned 5 mm above the interaural line. A cannula (22-gauge steel) was inserted into the lateral ventricle, through a hole drilled in the skull, 2 mm anterior and 2 mm lateral to the bregma; the tip was placed at a depth of 4 mm. The cannula was connected to a Hamilton microliter syringe by polyethylene tubing. Fifteen min after PB injections the rats received an injection of PrS (Steraloids; 8 μg/10 μl of 0.25% saline) into lateral ventricle. (0.25% saline was a better solvent for PrS than physiological 0.9%). Some animals received ICV injections of dehydroepiandrosterone sulfate (8 μg/10 μl/rat) or cholesterol sulfate (4 μg/10 μl/rat). A control group received 10 μl of 0.25% saline ICV.

For IP steroid treatment rats received PrS either 8.4, 12.6 or 16.8 mg/kg dissolved in 5 ml of 0.9% saline, pH 7.0, 15 min following PB injection. Control animals for this group received 5 ml of 0.9% saline IP.

The hypnosis time was measured from the moment of loss of righting reflex following PB injection until its return.

RESULTS

The typical onset time from the moment of PB injection until the loss of the righting reflex was 4–12 min. Rats that failed to fall asleep during 12 min following PB injection were discarded from

TABLE 1

EFFECT OF ICV-INJECTED PrS ON PENTOBARBITAL-INDUCED HYPNOSIS TIME IN SPRAGUE-DAWLEY RATS

		Hypnosis Time (min)	% of Reduction
Control	(n = 5)	108 ± 8	–
PrS	(n = 5)	70 ± 5*	35

Control rats received 10 μl of 0.25% saline ICV. PrS was injected at a dose 8 μg/10 μl (25% saline)/rat. The animals which failed to fall asleep during 12 min following injections of pentobarbital (45 mg/kg, IP) were discarded from experiments). Values represent means ± SEM; *Denotes values statistically different from control ($p<0.02$, n = 5; Student's *t*-test).

the experiments (about 20%). In control Sprague-Dawley rats, PB-induced hypnosis lasted for 108 ± 8 min (mean ± SEM; n = 5, Table 1), and PrS injected ICV (8 μg/10 μl/rat) reduced the sleep time to 70 ± 5 min (n = 5; $p<0.01$, Student's *t*-test). Dehydroepiandrosterone sulfate (ICV; 8 μg/10 μl/rat) and cholesterol sulfate (ICV; 4 μg/10 μl/rat) were without effect on time of PB-induced hypnosis (data not shown).

In Fischer-344 rats, PB-induced hypnosis lasted 144 ± 6 min (mean ± SEM; n = 8, Fig. 1). PrS injected IP reduced the hypnosis time in a dose-dependent manner (Fig. 1). At doses of 8.6, 12.6 and 16.8 mg/kg, PrS reduced the hypnosis time by 17%, 32% and 37%, respectively ($p<0.05$; n = 5 for PrS 8.6 mg/kg, and $p<0.01$, n = 5 for PrS 12.6 and 16.8 mg/kg; one-way ANOVA/Dunnett's test).

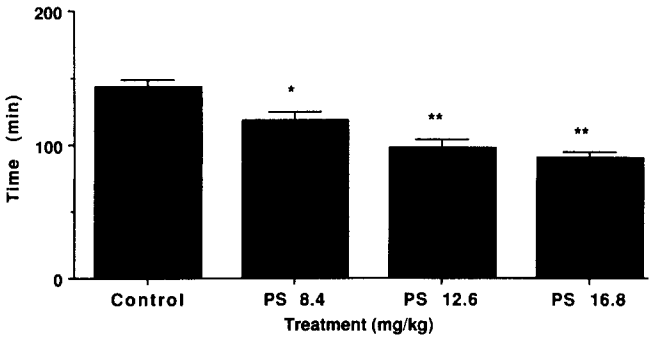

FIG. 1. Effect of IP-injected PrS on pentobarbital-induced hypnosis time. Fischer-344 rats received pentobarbital (45 mg/kg, IP). PrS was injected in concentrations 8.4, 12.6 and 16.8 mg/kg in 5 ml of 0.9% saline, pH 7.0 (for each group n = 5). Control rats received IP 5 ml of 0.9% saline (n = 8). Values represent means ± SEM; significantly different from control by $p<0.05$ (*) and $p<0.01$ (**) by one-way ANOVA and Dunnett's test.

PrS injected IP (12.6 mg/kg/3 ml saline) into awake Fischer-344 rats did not produce any gross behavioral effects during 1 hour following injection. However, since we did not test animals in these experiments in paradigms measuring animal correlates of anxiety, we cannot exclude possible anxiogenic effects of PrS. These studies are now in progress.

DISCUSSION

We have demonstrated that PrS, whether injected ICV or IP, markedly reduces PB-induced hypnosis time in rats. This observation is compatible with our earlier findings that PrS behaves in vitro as an antagonist of the $GABA_A$ receptor-operated chloride channel (8,9).

Since the mammalian body produces steroids (4, 6, 7, 14) which in vitro behave as allosteric agonists of the $GABA_A$ receptors (10) and in vivo manifest anesthetic/hypnotic/anxiolytic properties (2–4, 13) the current observation has important physiological implications. It is conceivable that the hypnotic steroids (reduced metabolites of progesterone and deoxycorticosterone), by enhancing the inhibitory function of $GABA_A$ receptors, may play a role in such states as sleep (13), stress, pregnancy (12), phases of menstrual or oestrus cycle, etc. On the other hand, PrS, which is produced in the CNS by the oligodendroglia (5), may counteract the effects of hypnotic steroids, by reducing the function of $GABA_A$ receptors. In vitro PrS indeed abolishes the Cl^- uptake-inducing effect of anesthetic steroids (8) and blocks electrophysiological responses to PB in neurons (9).

It is possible that the net, positive or negative modulation of $GABA_A$ receptor function in vivo may depend on a balance between hypnotic-anxiolytic steroids and PrS in the CNS. Hence, the abnormalities or individual variations in steroid-metabolizing enzymes may lead to differences in CNS excitability, with behavioral manifestations such as distinctive basal levels of arousal, sensitivity to hypnotics and others [see (11)]. Different individual sensitivity to anesthetics is, in fact, a known clinical phenomenon and in our experiments it was manifested by failure of about 20% of rats to fall asleep, following injection of 45 mg/kg of pentobarbital.

The current data complement our previous finding describing GABA-antagonistic properties of neurosteroid PrS (8,9) and provide evidence that PrS may act as an anesthetic antagonist in vivo. Our finding also has an obvious pharmacological implication suggesting that PrS (or analogs) could be used to counteract the effects of general anesthesia or hypnotic poisoning.

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