Potentiating and Delayed Effects of Dalargin Analogs on the Duration of Nembutal-Induced Sleep

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Simultaneous administration of Nembutal and the dalargin analog No. 3 prolongs duration of sleep in all rats and in 17% of mice by 25-450%. Administration of the dalargin analog No. 3 twenty-four hours before Nembutal results in a 2- to 3-fold increase in the duration of sleep compared with that after simultaneous administration of Nembutal and the analog.

Key Words: *sleep; opioid peptides; potentiating and delayed effects*

Some synthetic enkephalin analogs exhibit soporific activity [2,3]. A similar effect was described for other peptides, for example, cholecystokinin [7]. Enkephalins, dalargin, cholecystokinin, and their analogs do not cause sleep by themselves, but potentiate the effects of soporific agents [3,7]. The use of dalargin in combination with other drugs for general anesthesia during prolonged surgery allows a reduction in the dose of analgesic narcotics [3].

Peptides that shorten sleep, for example, vasoactive intestinal peptide, have been identified [1].

The ability of peptides to stimulate delayed effects has not been studied in detail. It was reported that Leu-enkephalin in combination with catecholamines and naloxone induces hypersensitivity of the mesenteric lymphatic microvessels to neutral stimuli 20 min and 2 h after administration [6]. Changes in the serotonin and tryptophan-5-hydroxylase levels in rabbit synaptosomes on day 5 after a single administration of the opioid tetrapeptides Tyr-D-Ala-Gly-Phe-NH_2 were demonstrated [4,5].

Our objective was to examine the effects of known and new dalargin analogs (DA) on the duration of Nembutal-induced sleep and to evaluate delayed effects of new peptides.

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MATERIALS AND METHODS

Experiments were performed on 120 male outbred and Wistar rats weighing 150-350 g and 800 albino mice weighing 10-30 g. The soporific effect of peptides was assessed by the duration of recumbency. Leu-enkephalin, enkephalin, bradykinin, dalargin and its analogs: Tyr-L-Ala-Gly-Phe-Leu-Arg (DA1), Tyr-D-Ala-Gly-Phe-Leu-Arg-NH_2 (DA2), and Tyr-D-Ala-Gly-Phe-Leu-Arg-NH-Et (DA3) were synthesized at the Laboratory of Peptide Synthesis of the Cardiology Research Center (Russian Academy of Medical Sciences). In rats, these peptides were applied onto the mesentery. In mice, they were injected intramuscularly in a dose of 0.004-400 μg/kg in 0.1 ml normal saline. Nembutal (sodium pentobarbital) was injected intramuscularly in doses 50, 75, and 100 mg/kg. Eight series of experiments (one on rats and seven on mice) were performed. Each series included 5-12 groups. Each group consisted of 10 animals. Control animals were injected with 0.1 ml normal saline, peptide, or Nembutal. Experimental animals were given Nembutal in combination with peptides. The effects of a 24-h pretreatment with Nembutal, peptides, and Nembutal-peptide combination were studied.

RESULTS

Application of all studied peptides and their analogs onto rat mesentery in doses 0.004-40 μg/kg 1 h after in-

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TABLE 1. Effects of Amino Acids, Oligopeptides, and Their Analogs on the Duration of Nembutal Sleep in Rats ($M\pm m$, n=10)

Compound	Duration of sleep, min	Student's test
Nembutal	178.22±12.24	—
Nembutal+Leu-enkephalin	203.66±12.90	1.380
Nembutal+Leu-enkephalin analog No. 1	169.11±20.65	0.379
Nembutal+Leu-enkephalin analog No. 2	193.25±10.59	0.928
Nembutal+dalargin	209.00±11.55	1.830
Nembutal+dalargin analog No. 1	201.25±20.95	0.950
Nembutal+dalargin analog No. 2	237.33±75.40	0.77
Nembutal+dalargin analog No. 3	229.00±14.64*	2.66
Nembutal+bradykinin	150.60±8.59	1.847
Nembutal+bradykinin analog	159.50±6.40	1.355
Nembutal+tyrosine	155.11±7.77	1.594
Nembutal+arginine	152.10±9.74	1.669

Note. Doses: Nembutal 100 mg/kg intramuscularly; peptides and amino acids 40 μg/kg application on the mesentery. *p<0.05 compared with the effects of Nembutal.

tramuscular injection of 100 mg/kg Nembutal changed the duration of Nembutal-induced sleep (Table 1). None of these peptides exhibited soporific activity in control animals (administration without Nembutal). Dalargin analog No. 3 (DA3) induced a 29% statistically significant increase in the duration of sleep in rats. Further experiments were performed on mice.

In mice, the duration of sleep increased in a dose-dependent manner (Fig. 1) except 50% of mice given the maximum dose of Nembutal (100 mg/kg). In these mice, the duration of sleep was 25% of that in other 50% of mice given the same dose of Nembutal. Administration of Nembutal in the same dose 24 h after the first one resulted in a decrease in reduced sleep duration (Fig. 1), except in the mice given the maximum dose. In these animals, the duration of sleep increased almost 2-fold: 181% of duration on day 1. Analysis of the data shown in Fig. 1 showed that an increase in the Nembutal dose leads to an increase in the degree of habituation. For example, the duration of sleep after the second administration of Nembutal was 58% of that after its first administration in a dose of 50 mg/kg, 34% after 75 mg/kg, and 29% after 100 mg/kg.

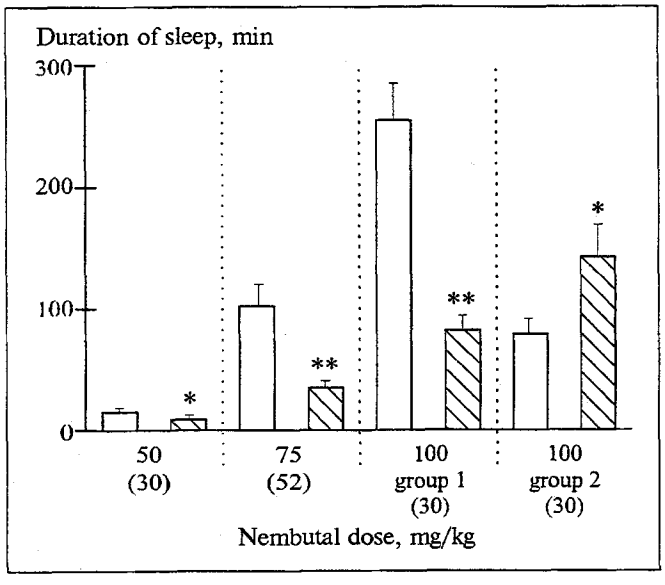

Fig. 1. Duration of sleep in mice after repeated administration of various doses of Nembutal. White bars: day 1; shaded bars: day 2. The number of animals is given in parentheses. *p<0.05; **p<0.0001 compared with day 1.

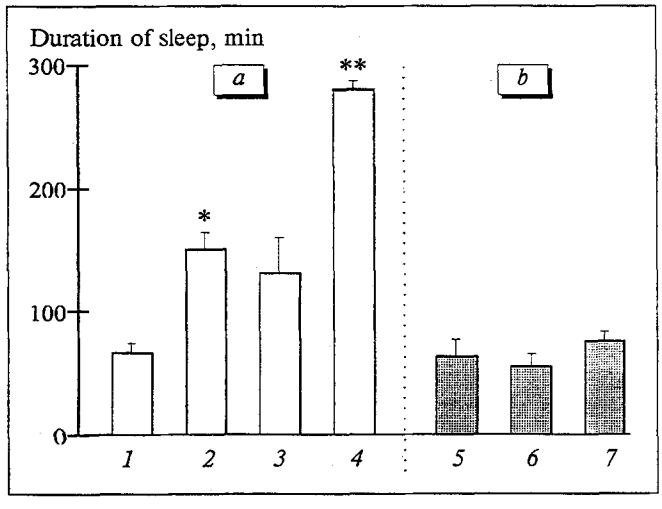

Fig. 2. Effects of dalargin analogs (DA) on the duration of Nembutal sleep in mice. *a*) duration of sleep after intramuscular administration of DA (40 μg/kg) with Nembutal (50 mg/kg) 24 h after administration of the same DA in a dose of 4 μg/kg; *b*) duration of sleep after a single intramuscular administration of DA (40 μg/kg) with Nembutal (50 mg/kg). *1*) Control with Nembutal; *2*, *5*) Nembutal with DA1; *3*, *6*) DA2; *4*, *7*) DA3. *p<0.05; **p<0.0001 compared with the control (*1*).

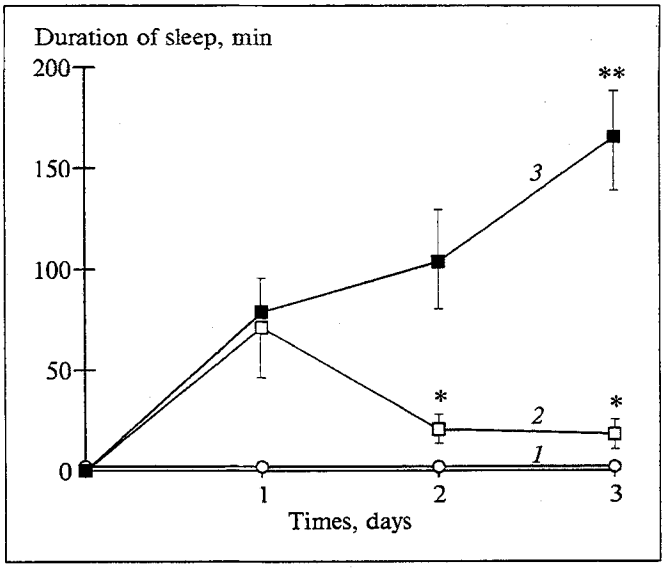

Fig. 3. Prolongation of Nembutal sleep in mice by dalargin analog No. 3 (DA3). *1*) DA3 (40 μg/kg); 2) Nembutal (75 mg/kg); *3*) Nembutal (75 mg/kg) with DA3 (40 μg/kg). *$p<0.01$, **$p<0.0001$ compared with day 1.

Administration of Nembutal (50 and 75 mg/kg) in combination with various doses of dalargin, DA1, or DA2 (4 and 40 μg/kg) had no effect on sleep duration in mice (n=65, $p>0.1$, Fig. 2, *b*). Administration of Nembutal with DA3 in the same doses did not prolong sleep in 99 out of 199 mice (83%) and prolonged it by 25-450% in the rest (17%, $p<0.05$ compared with the control). Prolonged administration of Nembutal and DA3 caused a marked increase in sleep duration in some animals. On days 2 and 3 of Nembutal (75 mg/kg) and DA3 (40 μg/kg) administration, sleep lasted 5- to 8-fold longer than after administration of Nembutal alone (Fig. 3). At the same time, prolongation of sleep was observed only on certain days of a 9-day-long treatment with Nembutal—DA3 combination (Fig. 4, *a*, *b*). This may be due to individual variations, since experimental conditions were identical. Seasonal variations did not affect the studied reaction.

After testing various combinations and doses of peptides with Nembutal, we chose the optimal schemes providing long and short sleep. On day 1, a peptide is injected intramuscularly in a small dose (4-40 μg/kg) which causes neither sleep nor any behavioral changes. On day 2, Nembutal is injected with the same dose or 10-fold higher dose of the peptide, but not higher than 400 μg/kg. The duration of sleep on day 2 increases 2- to 3-fold compared with that after administration of Nembutal alone (Fig. 2, *a*). Nembutal should be applied in a low dose (50 mg/kg, Table 2, Fig. 2, *a*), since the duration of sleep after this dose is the same as after 75 mg/kg. Increasing the Nembutal dose to 100 mg/kg seems unreasonable, since in combination with the peptide this dose reduces the duration of sleep.

Thus, administration of Nembutal and peptide in small doses prolongs the duration of sleep attained with high doses of Nembutal. Peptide partially substitutes Nembutal without reducing sleep.

Nembutal-induced sleep can be shortened by administering the peptides in high doses (Table 2). For example, administration of peptide on day 1 in a dose of 4 mg/kg followed by administration of Nembutal in 40 mg/kg peptide on day 2 provides the same or shorter sleep than that induced by Nembutal alone. This observation may be useful for the development of new schemes for termination of anesthesia.

Our results show that Leu-enkephalin analogs elicit delayed effects. These effects were observed 24 and 72 h after administration of small doses of the peptides as stimulation of activities of soporific agents. The duration of sleep can be regulated by varying the peptide dose. Dalargin analog No. 3, which elicited the highest potentiating effect, can be used in anesthesiology to reduce the dose of narcotic preparations and prevent complications caused by them, without changing the duration of sleep and anesthesia.

In contrast to the DA proposed previously for prolongation of Hexenal-induced sleep, a 250-fold lower dose of DA3 prolongs sleep by 5-10 times with-

TABLE 2. Delayed and Potentiating Effects of Dalargin Analog No. 3 (DA3) in mice ($M\pm m$, n=10)

Group No.	Day 1			Day 2		
	compound	dose, mg/kg	duration of sleep, min	compound	dose, mg/kg	duration of sleep, min
1	—	—	—	Nembutal	50	66.50±9.56
2	DA3	0.004	0	Nembutal+DA3	50+0.04	247.17±40.91*
3	—	—	—	Nembutal	75	71.30±19.02
4	DA3	0.04	0	Nembutal+DA3	75+0.04	143.50±20.60****
5	DA3	0.04	0	Nembutal	75	243.33±38.79*
6	DA3	4	0	Nembutal+DA3	75+40	37.80±9.40

Note. *$p<0.002$ compared with group 1; *$p<0.05$ compared with group 2; *$p<0.002$, **$p<0.02$ compared with group 3.

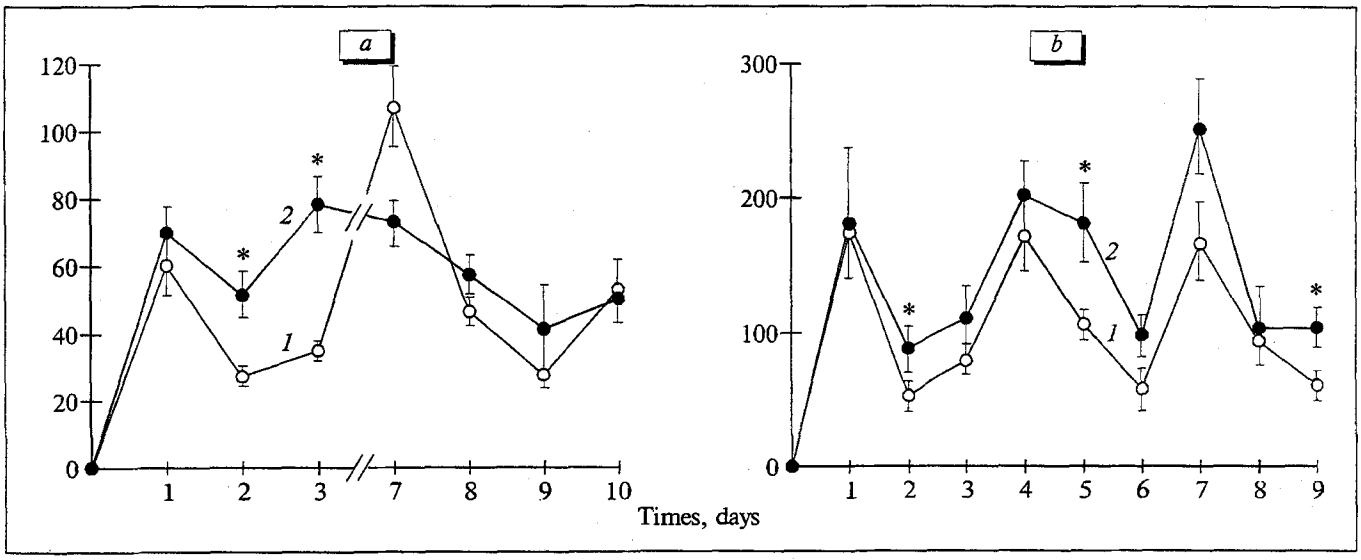

Fig. 4. Duration of sleep induced by various doses of Nembutal and DA3. *a*) February: Nembutal (75 mg/kg) and DA3 (400 μg/kg); *b*) May: Nembutal (100 mg/kg) and DA3 (40 μg/kg); *1*) Nembutal control; *2*) Nembutal with DA3. Ordinate: duration of sleep, min. *$p<0.01$ compared with the control.

out producing any toxic effect. Since none of the animals died, it can be concluded that LD_{50} for intramuscular administration is much higher than 400 mg/kg, which is 10,000-fold higher than the maximum effective dose. LD_{50} of the parental substance is 400 mg/kg.

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