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Thermoregulatory and Locomotor Effects of DSIP: Paradoxical Interaction with d-Amphetamine

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YEHUDA, S., A. J. KASTIN AND D. H. COY. *Thermoregulatory and locomotor effects of DSIP: Paradoxical interaction with d-amphetamine.* PHARMAC. BIOCHEM. BEHAV. 13(6) 895–900, 1980.—A low dose (0.1 mg/kg) of peripherally administered delta sleep-inducing peptide (DSIP) caused hypothermia in rats maintained at 4°C but larger doses (1.0 or 3.0 mg/kg) did not. At 22°C all 3 doses of DSIP caused hyperthermia. The interaction of d-amphetamine with DSIP was dependent on ambient temperature; at 4°C DSIP had no effect on d-amphetamine-induced hypothermia, but at 22°C DSIP reversed the usual d-amphetamine-induced effect of hyperthermia to hypothermia. At 4°C, DSIP also potentiated the hypermotility effects of d-amphetamine but blocked it at 22°C. Many rats receiving DSIP (0.1 mg/kg) and d-amphetamine (15.0 mg/kg) at 22°C paradoxically exhibited what appeared to be sleep. This sleep-like effect was not found after several other doses of DSIP and d-amphetamine, chlorpromazine, or at a cold ambient temperature. Sodium methohexital caused apparent sleep in animals treated with DSIP alone or with DSIP and d-amphetamine, but was unable to induce this effect in rats receiving d-amphetamine alone. The results suggest that the effects of peripherally injected DSIP on body temperature are independent from its effects on locomotion and that both effects are dependent upon ambient temperature.

Temperature Locomotion Peptide DSIP Sleep Amphetamine

ELECTRICAL stimulation of the rabbit thalamus can induce sleep. From the cerebral venous blood of these rabbits, a nine amino-acid peptide was isolated and called delta sleep-inducing peptide (DSIP) [9]. Administration of this peptide to awake animals causes an increase in delta (2–4 cps) waves which are associated with slow wave sleep and increased total sleeping time. Although not all researchers have been able to obtain these effects on sleep, several positive reports indicate that DSIP is able to induce delta waves in the majority of the tested animals, including rabbits [6,9]; cats [8], rats [1], and mice [7]. DSIP-like immunoreactivity can be found in rat brain [2], and it has been demonstrated that some DSIP can cross the blood-brain barrier [3].

During natural sleep, the body temperature of sleeping rats is decreased by as much as 1.8°C (Yehuda and Carasso, unpublished results). The question arises whether the effect of DSIP on sleep can be enhanced in rats made hypothermic by pharmacological manipulation. Exposure of rats to a cold ambient temperature (4°C) does not usually result in a decrease in body temperature [16]. Administration of d-amphetamine (5–15 mg/kg, IP) induces hyperthermia in rats maintained at room temperature or above (20°C–37°C), but induces hypothermia in rats kept at 4°C [17]. Several studies have indicated that the hyperthermic effects of d-amphetamine are partially mediated by release of dopamine in the dopaminergic mesolimbic pathway [10, 12, 13, 17, 18].

Some drugs which increase dopaminergic activity in the brain are able to produce hypothermia and to enhance the d-amphetamine-induced hypothermia in rats maintained at 4°C. Some other drugs which decrease dopamine, serotonin, or norepinephrine activity in the brain were shown not to block d-amphetamine-induced hypothermia [17]. Since the interaction of DSIP with brain neurotransmitters is not yet known, evaluation of the effects of DSIP on d-amphetamine-induced hypothermia might lead to better understanding of the interaction between DSIP and brain dopamine.

Behaviorally, the sleep state is obviously associated with a marked decrease of motor activity. Examination of the interaction between DSIP and d-amphetamine provides an opportunity to investigate the interaction of DSIP with a drug which causes hypothermia and hypermotility at 4°C and hyperthermia and hypermotility at room temperature. In this study, the effects of various doses of DSIP alone or in combination with d-amphetamine on sleep, thermoregulation, and motor activity were measured in rats kept at 4°C or at 22°C. In addition, the effects of the interaction of DSIP with chlorpromazine, which induces hypothermia and hypomotility at 4°C and 22°C, were examined. In order to further evaluate the effects of the treatments on sleep, various doses

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of DSIP alone or in combination with d-amphetamine were also studied in rats maintained at 22°C or at 4°C which had received the ultrashort anesthetic agent, sodium methohexital.

METHOD

Male Sprague-Dawley derived albino rats (Blue Spruce Farms, Altamont, NY) weighing 90–120 grams were housed, 6 per cage, in a well ventilated room, at an ambient temperature of 22°C. They had free access to food and water. Lights were on from 6:00 to 18:00 hours daily.

Just before each experiment, rats were placed in individual cages, and, after a few minutes, colonic temperature was measured at 6 cm with a telethermometer (Yellow Spring Inst., Yellow Spring Corp, OH). Each rat then received an intraperitoneal (IP) injection of either physiological saline (0.9% NaCl) or DSIP (0.1, 1.0, or 3.0 mg/kg), and was placed in a chamber preset to 4°C or to 22°C. Colonic temperatures were measured every 15 min for 2 hrs.

In the double-injection experiment, each rat received an IP injection of DSIP (0.1, 1.0, or 3.0 mg/kg) and was placed in the environmental chamber preset to 4°C or to 22°C. Thirty min later, each rat received an injection of d-amphetamine (15 mg/kg), chlorpromazine (10 mg/kg), or saline.

Levels of locomotion were measured 30 min after the first injection, and every 30 min thereafter for 2 hrs by an observational rating scale ranging from −2 (below the level (0) of activity in saline-treated rats) to +4.

Sleep-like behavior was evaluated by an observational method consisting of the following parameters: immobility, loss of muscle tone with lying on the side, half-closed eyes, regular slow breathing, and lack of response to photic and acoustical stimuli.

Other rats were individually placed in a cage and given one dose of DSIP (0.1 or 1.0 mg/kg) or saline. After 30 min, half of each treated group received 15 mg/kg d-amphetamine or saline; 45 min after the first injection all rats received 60 mg/kg sodium methohexital (Sodium Brevital, Lilly), and the latency to sleep and length of sleep were measured. In each experimental group there were at least 12 rats tested in groups of 6 on different days. All experiments were performed between 10:00 to 14:00 hours.

RESULTS

The low dose of DSIP (0.1 mg/kg) induced significant ($p<0.01$) hypothermia and hypomotility ($p<0.01$) in rats maintained at 4°C. However, the magnitude of the hypothermic response was smaller than that induced by d-amphetamine (15.0 mg/kg). Pretreatment with this dose of DSIP did not modify the magnitude of d-amphetamine-induced hypothermia (Fig. 1). In contrast to the lack of effect of DSIP on d-amphetamine-induced hypothermia at 4°C, pretreatment with 0.1 mg/kg DSIP increased ($p<0.01$) the hyperactivity induced by d-amphetamine (Fig. 1).

The effects of larger doses of DSIP (1.0 or 3.0 mg/kg) were similar to each other for both thermoregulation and motor activity. Neither dose had any effect on the body temperature of rats maintained at 4°C nor did they not modify the magnitude of d-amphetamine-induced hypothermia. They also did not cause hypomotility but they did enhance d-amphetamine-induced hypermotility ($p<0.05$). The results of the effects of 1.0 mg/kg DSIP are presented in Fig. 2 and the results with 3.0 mg/kg were almost identical.

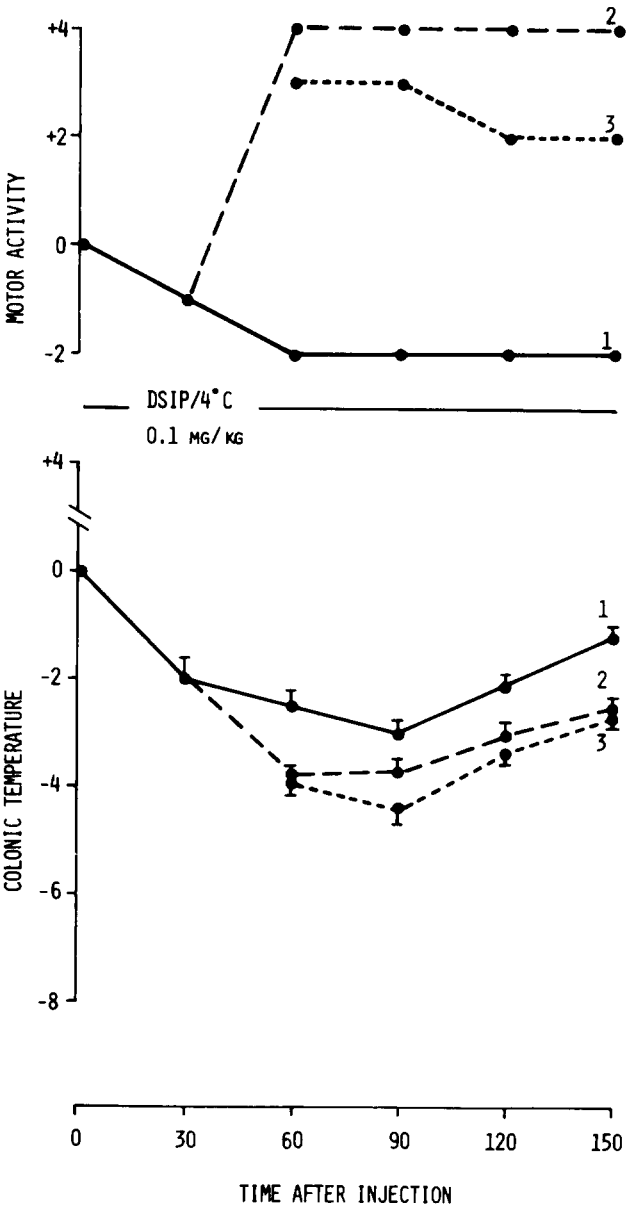

FIG. 1. Effects of 0.1 mg/kg DSIP with or without 15 mg/kg d-amphetamine on locomotion (upper part) and on colonic temperature (lower part) in rats maintained at 4°C. Results are expressed as changes from initial locomotion and changes from initial colonic temperature. Each dot represents the mean of at least 12 animals. Vertical bar represents S.D. 1=DSIP alone, 2=DSIP+d-Amphetamine, 3=d-Amphetamine alone.

Different effects of the various doses of DSIP were observed when the animals were maintained at 22°C. A dose of 0.1 mg/kg DSIP induced significant hyperthermia (mainly 60–150 min after injection, $p<0.01$). This hyperthermia was smaller than that induced by 15 mg/kg d-amphetamine. In spite of the hyperthermic effects of each drug given alone at 22°C, the combination of DSIP and d-amphetamine at 22°C produced a dramatic hypothermic effect (Fig. 3). Pretreatment with 0.1 mg/kg DSIP alone also reversed the d-amphetamine-induced hypermotility, and most of the rats

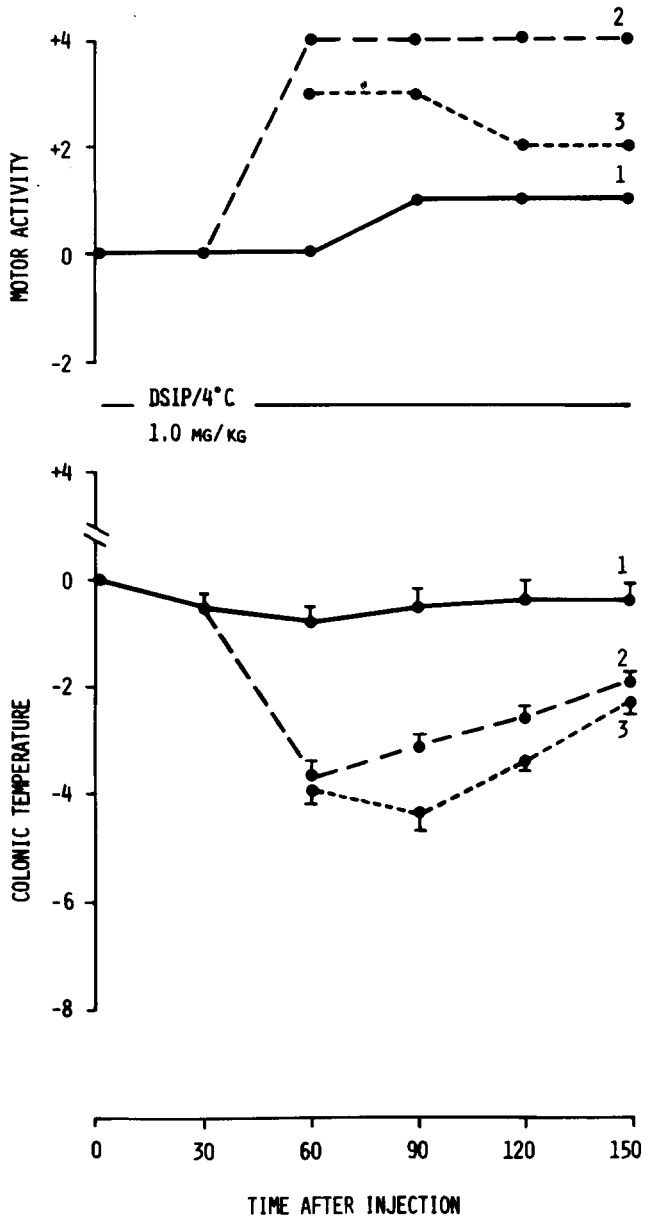

FIG. 2. Effects of 1.0 mg/kg DSIP on locomotion (upper part) and on colonic temperature (lower part) in rats maintained at 4°C. Results are expressed as changes from initial locomotion and changes from initial colonic temperature. Each dot represents the mean of at least 12 animals. Vertical bar represents S.D. 1=DSIP alone, 2=DSIP+d-Amphetamine, 3=d-Amphetamine alone.

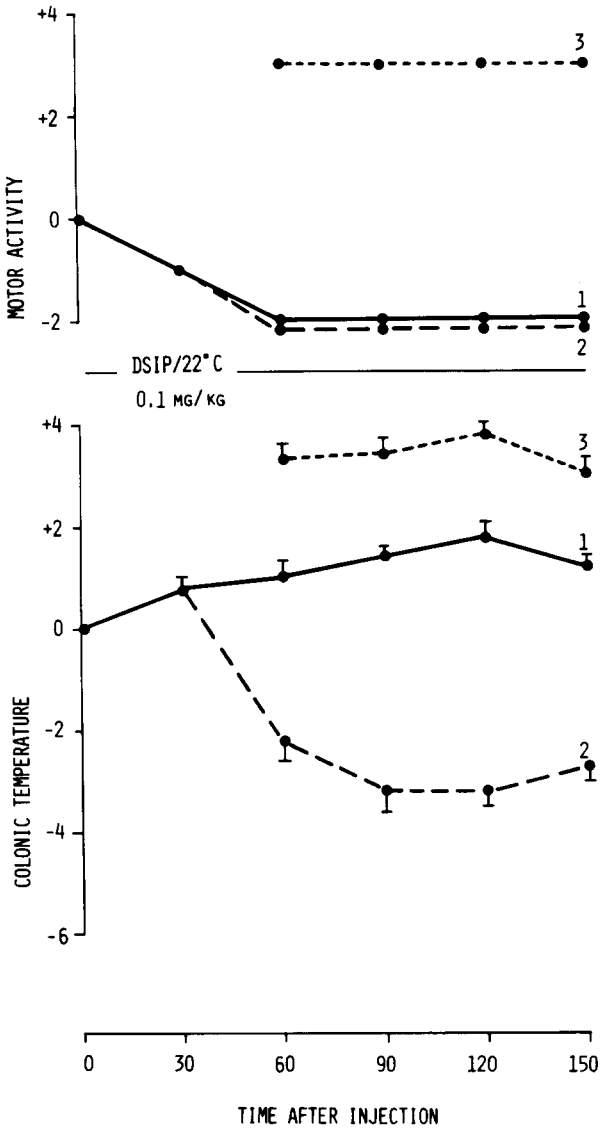

FIG. 3. Effects of 0.1 mg/kg DSIP on locomotion (upper part) and on colonic temperature (lower part) in rats maintained at 22°C. Results are expressed as changes from initial locomotion and changes from initial colonic temperature. Each dot represents the mean of at least 12 animals. Vertical bar represents S.D. 1=DSIP alone, 2=DSIP+d-Amphetamine, 3=d-Amphetamine alone.

(19 out of 24) exhibited behavior resembling sleep; i.e., their eyes were half closed, they were lying on their side or curled, breathing was very regular and slow, and they did not react to acoustical and photic stimuli during this period of time which started about 30–40 min after the injection of 15 mg/kg d-amphetamine.

Larger doses of DSIP (1.0 or 3.0 mg/kg) exerted the same effects as the smaller dose at 22°C, but despite the reversal of d-amphetamine induced hypermotility, no rat exhibited sleep-like behavior. The effects of 1.0 mg/kg, which were practically indistinguishable from those of 3.0 mg/kg, are shown in Fig. 4. Administration of DSIP alone, at any of the doses tested, did not result in sleep. The apparent sleep-inducing effect of the combination of d-amphetamine and DSIP seemed to occur with only one of the doses of d-amphetamine tested, i.e., 15 mg/kg. Administration of a smaller dose of d-amphetamine (5 mg/kg) to rats pretreated with DSIP (0.1, 1.0, or 3.0 mg/kg) did not result in sleep-like behavior but rather in potentiation of d-amphetamine-induced hypermotility at both ambient temperatures (4°C and

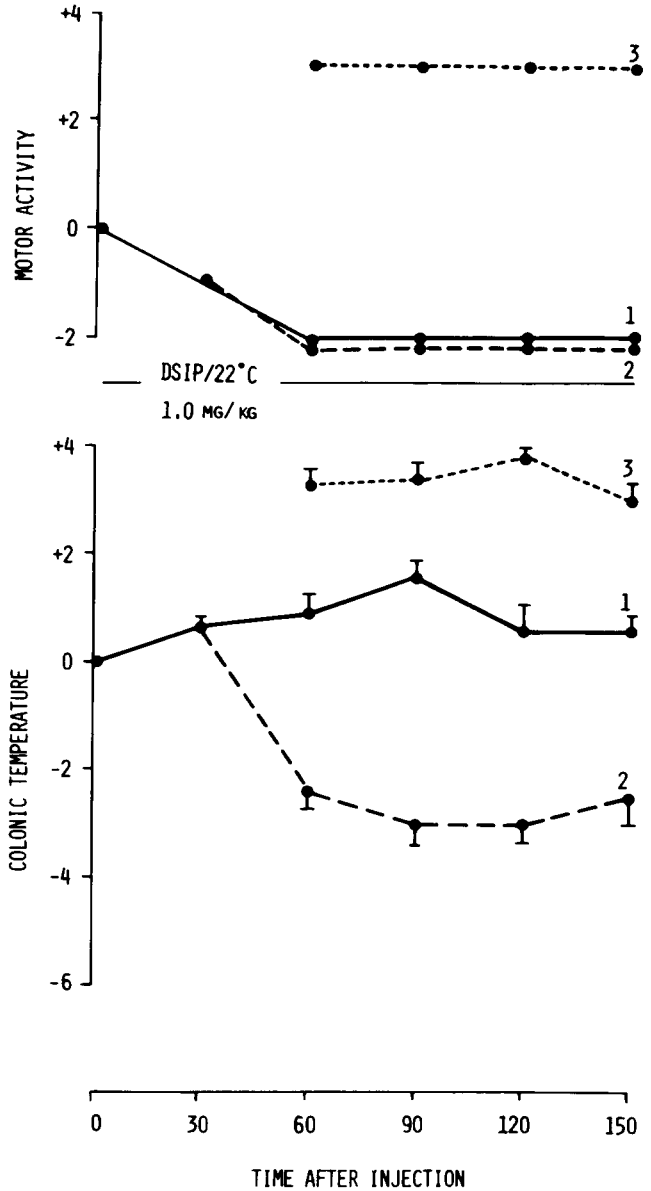

FIG. 4. Effects of 1.0 mg/kg DSIP on locomotion (upper part) and on colonic temperature (lower part) in rats maintained at 22°C. Results are expressed as changes from initial locomotion and changes from initial colonic temperature. Each dot represents the mean of at least 12 animals. Vertical bar represents S.D. 1=DSIP alone, 2=DSIP+d-Amphetamine, 3=d-Amphetamine alone.

22°C). In addition, administration of 10 mg/kg chlorpromazine to rats pretreated with DSIP (0.1, 1.0, or 3.0 mg/kg) did not modify the thermal or motor effects of DSIP at either 4°C or at 22°C. Moreover, no dose of DSIP (0.1, 1.0, or 3.0 mg/kg) modified the effects of 15 mg/kg d-amphetamine when the injection of d-amphetamine preceded that of DSIP in the same schedule.

Administration of 60 mg/kg sodium methohexital to rats maintained at 22°C induced sleep-like behavior lasting for 13 min after a latency of 1 min. Pretreatment with DSIP (0.1, 1.0 or 3.0 mg/kg) did not modify the effects of sodium methohexital, but pretreatment with 15 mg/kg d-amphetamine caused total inhibition of the apparent sleep-inducing effect of the drug. However, when rats were pretreated with a combination of DSIP and d-amphetamine, sodium methohexital was able to induce sleep-like behavior in 9 out of 12 tested rats. No significant modifications in latency or duration of apparent sleep were observed when sodium methohexital was given alone, with DSIP, or with DSIP plus d-amphetamine (Table 1). When the sleeping animals awoke, they displayed a very high level of motor activity, but instead of walking they hopped with quick, jerky movements and were extremely reactive to photic and acoustical stimuli. In the remaining 3 non-sleeping rats, long periods of convulsions were observed, raising the possibility that all the sleep-like effects represented a form of toxicity. Although sodium methohexital induced significantly longer sleep-like activity (18 min, $p<0.05$) in rats maintained at 4°C than at 22°C, no alteration in the apparent sleep was observed at either temperature when sodium methohexital was given to rats pretreated with DSIP alone or with DSIP and d-amphetamine.

DISCUSSION

As previously reported, 15 mg/kg d-amphetamine caused hyperthermia in rats maintained at 22°C, and hypothermia in rats maintained at 4°C. A dose of 10 mg/kg chlorpromazine induced hypothermia at both ambient temperatures [11,13].

The thermal effects of DSIP are unusual. A low dose of DSIP (0.1 mg/kg) induced hypothermia at 4°C, whereas all tested doses of DSIP caused an increase in body temperature at 22°C. Most of the other peripherally administered peptides cause hypothermia or have no effect on the body temperature of rats tested at room temperature [14]. Also, the reversal of the hyperthermic effects of d-amphetamine by pretreatment with DSIP is unusual. Although pretreatment of rats with MIF-I [Pro-Leu Gly-NH_2] or alpha-melanocyte-stimulating hormone (alpha-MSH) resulted in a decreased magnitude of d-amphetamine-induced hyperthermia [15], no reversal of the hyperthermic effect of d-amphetamine has been reported for any other peptide.

Low doses of DSIP (0.1 mg/kg) induced hypomotility in rats maintained at ambient temperatures of 4°C and at 22°C, but larger doses (1.0 or 3.0 mg/kg) induced hypomotility only among rats maintained at an ambient temperature of 22°C. Despite the significant decrease in locomotion, rats treated with DSIP did not exhibit signs of sleep. Their eyes were wide open, and they reacted to various photic and acoustical stimuli. The effects of d-amphetamine (15.0 mg/kg) on DSIP-treated rats were dependent on ambient temperature. Although potentiation by DSIP of the hypermotility-effect of d-amphetamine was observed in animals tested at 4°C, significant blocking of the hypermotility-effect by DSIP was found when the animals were tested at an ambient temperature of 22°C. Administration of 15 mg/kg d-amphetamine to rats pretreated with 0.1 mg/kg DSIP and kept at an ambient temperature of 22°C resulted, paradoxically, in apparent signs of sleep or perhaps narcosis, i.e., loss of muscle tone, half closed eyes, and a lack of response to environmental stimuli. This type of paradoxical effect of d-amphetamine on motor activity is somewhat similar to the paradoxical calming effect of the drug on hyperkinetic children. However, the paradoxical effect associated with d-amphetamine was not found with other doses of DSIP or at other ambient temperatures.

The results of this study indicate that the relationship between sleep-like behavior and decreased body tempera-

TABLE 1

APPARENT SLEEPING TIME AFTER VARIOUS TREATMENTS

Treatment	DSIP 0.1 mg/kg		DSIP 1.0 mg/kg	
	Latency	Total sleeping time (sec)	Latency	Total sleeping time (sec)
Brevital	49.0 ± 2.0	753.5 ± 190.6	49.4 ± 21.0	765.0 ± 182.4
DSIP	no sleep	no sleep	no sleep	no sleep
d-Amp	no sleep	no sleep	no sleep	no sleep
DSIP + Brevital	51.2 ± 13.0	856.1 ± 40.8	69.0 ± 36.0	894.4 ± 51.6
d-Amp + Brevital	no sleep	no sleep	no sleep	no sleep
DSIP + d-AMP + Brevital	88.6 ± 76.0	784.0 ± 99.0	99.0 ± 40.0	803.1 ± 104.5

The effects of sodium methohexital (Brevital) (60 mg/kg), DSIP (0.1 or 1.0 mg/kg), d-Amphetamine (d-Amp) (15 mg/kg), alone or in combination on latency to apparent sleep (sec) and on total sleep-like time (sec) of rats maintained at 22°C. Data expressed as a mean and SD of at least 12 rats in each experimental group.

ture are more complex than was assumed. A decrease in body temperature or exposure to a cold ambient temperature did not promote sleep. Also, chlorpromazine, which causes both hypothermia and loss of muscle tone, was unable to promote sleep when given alone or in combination with DSIP. The possibility that the hypermotility induced by the combination of d-amphetamine and cold prevented sleep-like behavior after DSIP is unlikely since rats also exposed to cold and DSIP but given the hypomotility-inducing drug chlorpromazine also did not sleep.

The mechanism by which d-amphetamine paradoxically promotes the apparent sleep-effect of low doses of DSIP is not known. Several hypotheses could be offered (e.g., d-amphetamine may increase the passage of DSIP across the blood-brain barrier or release another neurotransmitter like serotonin which promotes slow wave sleep), but speculation about the mechanism is premature at this time.

The finding that a particular dose of d-amphetamine (15 mg/kg) was able to promote sleep-like behavior in combination with a particular dose of DSIP (0.1 mg/kg) among animals tested at a particular ambient temperature (22°C) but not in some other experimental conditions is not surprising. A non-linear dose-response relationship for the effects of many peptides has been frequently described [4]. Recently, we were able to show that the dose-response curve for the thermal effects of peripherally administered beta-endorphin is also not linear [14].

Whatever the mechanism, the effect of d-amphetamine and DSIP on sleep-like behavior seems paradoxical. When given alone, d-amphetamine blocked the apparent sleep-inducing effect of sodium methohexital, but it was unable to exhibit this effect when given with DSIP. Perhaps 4°C is too "stressful" for rats to sleep.

The finding that pretreatment with DSIP did not modify d-amphetamine-induced hypothermia at 4°C, indirectly indicates that DSIP does not primarily interact with dopaminergic neurons. Also, the results of this study strongly support the concept that thermoregulation and motor activity are under separate control systems, since it was found that the combined effect of DSIP and amphetamine on thermoregulation is independent from the effect on locomotion. A similar dissociation was reported between the effects of beta-endorphin on body temperature and locomotion [15].

Recently it has been suggested that DSIP might exert more effects than just promoting appearance of the delta waves [5]. The results of the present study indicate that under certain experimental conditions various doses of DSIP have significant effects on thermoregulation and locomotion.

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