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SUBSTANCE P FOUND TO LOWER BODY TEMPERATURE AND AGGRESSION

Edward T. Uyeno[1], Ding Chang[2], and Karl Folkers[2]

[1]Life Sciences Division, SRI International
Menlo Park, California 94025

[2]Institute for Biomedical Research
The University of Texas at Austin
Austin, Texas 78712

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SUMMARY

Synthetic substance P (SP) was bioassayed in mice by a procedure comprising eleven subtests. Two replications of a dose-response study were conducted at the time of the peak effect of the intravenous injection of SP. Single doses of 31 and 63 ng/kg significantly decreased body temperature. SP, [D-Arg^1]-SP, and [des-NH_2-Arg^1]-SP comparably lowered blood pressure, but [D-Arg^1]-SP and [des-NH_2-Arg^1]-SP were 1/20 to 1/10 as active as SP in lowering body temperature. The activities that lower body temperature and blood pressure may be different. The thyrotropin and luteinizing hormone releasing hormones (TRH and LH-RH) did not lower body temperature. SP also decreased the aggressive response (ED_{50}, 89 ng/kg).

A monograph (1) published in 1976 provided a comprehensive survey on the research that had been conducted on Substance P (SP) since von Euler and Gaddum detected it in 1931 (2). Now that synthetic SP (H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) is available, research on the physiological roles of this polypeptide neurotransmitter has been broadened and intensified.

Relatively little research has been conducted on the behavioral effects of SP, however. Early experiments were conducted with crude extracts of the natural peptide from tissues, which showed that it had tranquilizing effects in mice (3), rats (4), rabbits (5,6), wild hares (7), and Betta splendens (8). Stern and Hadzović (9) reported that intramuscular injections of 250 μg/kg synthetic SP tranquilized aggressive mice for at least 180 minutes. Stewart et al. (10) found that intracerebral injections of 2ng/mouse produced analgesia for more than 90 minutes.

To increase the knowledge about the activities of synthetic SP, we bioassayed the undecapeptide in a primary mouse screen that comprises a battery of physiological and behavioral subtests.

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METHODS

Male Swiss-Webster mice weighing 18 to 20 g, supplied by Simonsen Laboratories, Inc. (Gilroy, California), were used. To control for the initial intersubject variation in responses to several subtests, we applied an experimental design wherein each mouse served as its own control. Specifically, locomotor activity, touch response, aggression, and neuromuscular coordination tests were administered before treatment to obtain baseline scores as a basis for comparing the predrug and postdrug responses of the mice. SP was dissolved in 0.9% sterile saline, and the solution was injected intravenously in either of the lateral tail veins at the standard volume of 10 ml/kg and at the uniform rate of 0.05 ml/sec.

Initially, an experiment on the time of peak effect was conducted with 60 mice assigned equally to three experimental groups (I, II, and III) and three control groups (A, B, and C). The treated animals were administered 0.25 mg/kg SP; the control subjects were given the vehicle. Group I and Group A were tested 2 to 8 min after injection. Groups II and B were observed 15 to 21 min after treatment. Groups III and C were examined 30 to 36 min after treatment.

After determining peak time, we conducted a dose-range experiment to find the minimum dose range at which a substantial percentage of treated animals showed an appreciable change in response in any of the subtests. On the basis of the dose-range study, SP doses of 0.016, 0.031, and 0.063 mg/kg (10 animals/dose) were selected for a dose-response study. The experimental and control animals were tested at the time of peak effect. The dose-response study was replicated several months later.

In each experiment, the test procedure was as follows. The undisturbed behavior of each animal was observed to detect any behavioral abnormality. Each animal's eyes were closely inspected for any irregularities. Then the subject was tested in an open-field box (61 X 61 X 45 cm) with a black floor, delineated with white paint to form 64 squares, each 7.6 sq cm (11). Postural arrest was noted when the mouse, placed at the center of the box, took a few steps, stopped abruptly, and remained motionless for more than 10 sec. Locomotor activity was scored by counting the total number of lines the animal crossed during the 2-min test period. After the test session, a sound was emitted by means of a metal clicker held at the top of the open box; the absence or presence of startle responses was noted. The touch response was evaluated by watching the animal's reaction when its thorax was touched with the tip of a long forceps. To test for aggressive

behavior, the animal was held at the nape of the neck, and a small wooden dowel was brought against its mouth to observe its biting response (12). Body temperature was registered by a rectal probe connected to a telethermometer. Neuromuscular coordination was measured by a rotarod revolving at the rate of 15 rpm (13); the duration of time that the mouse was able to stay on the rotarod during the 2-min test period was recorded. Muscle tone was assessed by allowing the animal to suspend from a tiny bar fixed to a Chatillon scale and slowly pulling the mouse down by its tail; the amount of force required to compel it to release its grip was read from the gauge. In the analgesic test, the animal was placed on a hot plate regulated at 55° ± 0.5° C (14). The time the animal was able to remain on the plate without showing reaction to heat was recorded.

RESULTS AND DISCUSSION

The subtest score of each treated mouse was considered to show a decrease (-), no change (0), or an increase (+) in response to a predrug baseline or to a control value. The control mice injected intravenously with 0.9% sterile saline solution and tested 2 to 30 min later had body temperatures ranging from 36.5° to 37.5°. Therefore, for the all-or-none type of data analysis, a treated animal that had body temperature lower than 36.5° was considered to have shown a decrease; a temperature above 37.5° was considered to be an increase. If an animal that had bitten the dowel during the predrug test did not bite it during the drug test, it was considered to have shown a decrease in aggression.

Table 1 summarizes the results of the peak time experiment. A considerable number of treated animals showed a reduction in body temperature. The highest percentage of mice that had reduced body temperature were in the group tested 2 to 8 min postinjection; that group also had the greatest proportion of nonbiters. Therefore, the time of peak effect of SP was considered to be 2 to 8 min after intravenous administration. The influences of SP had dissipated in most subjects 30 min after the injection.

Table 2 presents the data obtained from the dose-response studies. At 0.031 and 0.063 mg/kg, SP produced hypothermic effects in high percentages of animals. A statistical test of significance of the results of Experiment 1 indicated that the mean body temperature (36.2°) of the group injected with 0.063 mg/kg SP and that (36.3°) of the group treated with 0.031 mg/kg were significantly lower than the mean (37.1°) of the control group ($t = 4.62$, $df = 18$, $p < .001$) $t = 4.55$, $df = 18$, $p < .001$, respectively). The mean

Table 1
THE TIME OF PEAK EFFECT EXPERIMENT
PERCENTAGE OF ANIMALS SHOWING A DECREASE (-), NO CHANGE (0), OR AN INCREASE (+) IN RESPONSE

Subtests	Effects of 0.25 mg/kg Substance P		
	Postinjection Time (min)		
	2 to 8	15 to 21	30 to 36
Behavioral abnormalities	0	0	0
Eye examination	0	0	0
Locomotor activity	(-) 20	(-) 10	(-) 10
Postural arrest	(+) 30	(+) 30	(+) 20
Startle response	0	0	0
Touch response	0	0	0
Aggression	(-) 70	(-) 40	(-) 10
Body temperature	(-)100	(-) 50	(-) 20
Coordination	0	0	0
Muscle tone	0	0	0
Analgesia	(+) 20	(+) 20	(+) 10

Table 2
DOSE-RESPONSE STUDIES
PERCENTAGE OF ANIMALS SHOWING A DECREASE (-) NO CHANGE (0), OR AN INCREASE (+) IN RESPONSE

Subtests	Experiment 1			Experiment 2		
	Group Treated with Substance P (mg/kg)			Group Treated with Substance P (mg/kg)		
	0.016	0.031	0.063	0.016	0.031	0.063
Behavioral abnormalities	0	0	0	0	0	0
Eye examination	0	0	0	0	0	0
Locomotor activity	(-) 10	(-) 10	(-) 10	0	0	0
Postural arrest	(+) 10	(+) 10	(+) 10	0	(+) 10	(+) 10
Startle response	0	0	0	0	0	0
Touch response	0	0	0	0	0	0
Aggression	(-) 20	(-) 30	(-) 40	(-) 10	(-) 20	(-) 40
Body temperature	(-) 40	(-) 80	(-) 90	(-) 30	(-) 70	(-) 90
Coordination	0	0	0	0	0	0
Muscle tone	0	0	0	0	0	0
Analgesia	0	0	0	0	0	0

temperature (36.7°) of the group given the lowest dose was not significantly different from that of the control group ($t = 2.03$, $df = 18$, $.05 < p < .10$).

To measure the significance of the effect of SP on aggressive behavior, the χ^2 test of independence was applied (15). The nonparametric test shows

that the two variables are not independent, indicating that SP significantly decreased aggression ($\chi^2 = 8.366$, df = 1, $p < .01$).

Results of the temperature and aggression tests of Experiment 1 were further analyzed to determine the median effective dose (ED_{50}), defined as an estimated dose that will cause a change in physiological state (e.g., body temperature) or in behavioral response (such as aggression) in 50% of the animals. According to the probit method of Litchfield and Wilcoxon (16), the ED_{50} that reduced body temperature and the ED_{50} that decreased aggression were 0.019 (0.012 - 0.029) mg/kg and 0.089 (0.039 - 0.205) mg/kg, respectively.

Comparable results were found in Experiment 2. The mean body temperature (36.3°) of the group treated with 0.063 mg/kg SP and that (36.4°) of the group injected with 0.031 mg/kg were significantly lower than the mean (37.0°) of the control group ($t = 4.65$, df = 28; $p < 0.01$; $t = 4.34$, df = 28, $p < .01$, respectively). The mean body temperature (36.7°) of the group given 0.016 mg/kg was not significantly different from that of the control group ($t = 1.89$, df = 28, $p > .05$ but $p < .10$). The median effective dose calculated from the results of the body temperature examination and the ED_{50} computed from the analysis of the aggression test were 0.022 (0.014 - 0.035) mg/kg and 0.09 (0.041 - 0.20) mg/kg, respectively. These results are almost identical with those of the first experiment.

To our knowledge, this is the first report that SP decreases temperature of mice. The finding that SP decreased aggressive behavior of mice confirms the results of Stern and Hadzović (9), who reported that synthetic SP inhibited isolation-induced aggression in mice. Interestingly, intravenously-injected SP had little or no effect on analgesia at the doses used.

The two analogs of SP, [D-Arg^1]-SP and [des-NH_2-Arg^1]-SP, were subsequently compared with SP for their activities in lowering body temperature. The ED_{50}s in mg/kg were: 0.02 for SP, 0.26 for [D-Arg^1]-SP, and 0.343 for [des-NH_2-Arg^1]-SP. Thus, the analogs were considerably less effective than SP in lowering body temperature by a factor of about 10 to 20. In contrast, Professor Sune Rosell, Karolinska Institutet, Stockholm, has found that these two analogs showed the same activity and potency as SP in lowering blood pressure when they were injected into dogs anesthetized by nembutol. Although small doses of SP and the two analogs reduced body temperature, relatively high doses (1, 2, and 4 mg/kg) of thyrotropin-releasing hormone (TRH) and 8 mg/kg luteinizing hormone-releasing hormone (LH-RH) had no significant effects on body temperature of the mouse.

Our data are insufficient to determine whether our results in mice may be species specific or may represent drug side effects. However, SP is known to cross the blood-brain barrier (9) and to occur naturally in the hypothalamus. Accordingly, an interesting speculation is that the lowering of body temperature by SP might be due to its action within the hypothalamus (17-18), which has been presumed to control body temperature (19-23). Clearly the effects of SP on body temperature and the mechanisms involved merit further investigation.

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