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BRIEF COMMUNICATION

Impaired Escape Reaction From Noxious and Nonnoxious Heat in Rats Treated With the Selective Noradrenergic Neurotoxin DSP-4

M. HAJÒS AND G. ENGBERG*

Department of Physiology, A. Szent-Györgyi Medical University, H-6720 Szeged, Dòm tèr 10, Hungary
**Department of Pharmacology, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden*

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HAJÒS, M. AND G. ENGBERG. *Impaired escape reaction from noxious and nonnoxious heat in rats treated with the selective noradrenergic neurotoxin DSP-4.* PHARMACOL BIOCHEM BEHAV **39**(3) 809–811, 1991.—The escape reaction, as an indicator of behavioral thermoregulation, was studied in rats pretreated with the selective noradrenergic neurotoxin DSP-4. The animals were kept for 30 min in a heated floor (42, 44, 46, 48 and 50°C) cage containing a wooden platform placed at a height of 12 cm, which enabled the rats to escape from the warm floor. The latency of escape and the time spent on the platform were recorded. The performance of DSP-4-treated rats was significantly inferior to that of the control rats at all tested temperatures. These findings indicate a general function of the central noradrenergic neurons in defense alarm reactions, including a significant role in heat defense behavior.

Escape reaction Behavioral thermoregulation DSP-4 Locus coeruleus Noradrenaline

THE majority of noradrenaline (NA) neurons in the rat brain are located in the locus coeruleus (LC). This pontine nucleus gives rise to a broad efferent network, and the neuronal activity is strongly correlated with arousal and vigilance and responds to a variety of external sensory stimuli (1, 5, 6, 12, 15). Previous electrophysiological studies have shown that LC neurons in anesthetized animals are activated by peripheral nonnoxious or noxious heat stimulation (4,9). This activation is prevented, however, by selective lesion of the primary sensory C-afferents by means of capsaicin (8); such treatment also results in a heat defense inability, i.e., capsaicin-treated rats at a warm ambient temperature are unable to control their body temperature as a consequence of an imperfect heat sensation (10, 13, 14, 16). The serious impairment of the behavioral thermoregulation and the absence of the LC response to peripheral warm stimulation in capsaicin-treated rats prompted us to investigate directly a putative role of the NA neurons in behavioral thermoregulation by using N-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4), a selective NA nerve cell neurotoxin.

METHOD

Male Wistar rats weighing between 200 and 250 g were used throughout the experiments. The animals were kept under standard laboratory conditions with free access to food and water. DSP-4 (Astra Lakemedel, Sweden) was dissolved in physiological saline and administered intraperitoneally. Rats were injected with 200 μmol/kg (63 mg/kg) of DSP-4 or saline twelve days before the experiments. According to the previous biochemical experiments, this dose of the drug causes a permanent loss of central NA, with only a temporary decrease in the peripheral NA level (3, 11, 17).

The heat defense behavior test, as an indicator of behavioral thermoregulation, was performed as described previously (13). Briefly, the escape reactions of the rats from different temperatures were determined. Each rat was placed in a cage ($24 \times 24 \times 50$ cm) with an open roof and a metal floor, which was heated by means of an ultrathermostat device. A wooden platform (6×24 cm), placed at a height of 12 cm, enabled the rat to escape from the warm floor. During the experiments, the floor of the cage was heated in a random sequence to 42, 44, 46, 48 or 50°C. The animals were kept in the cage for 30 minutes, and the time spent on the platform and the latency of escape were determined. In some experiments, the body temperature was also recorded by means of a thermistor probe (Yellow Springs Inst., USA) inserted 6 cm into the rectum of the rats under light manual restraint. The body temperature was recorded before and immediately after the escape test.

In a separate experiment, the sensitivity to heat pain was tested with a hot-plate procedure at 56°C. The time from contact with the floor until a hind-paw lick occurred was recorded as the response latency. For the evaluation of heat tolerance following DSP-4 treatment, control and treated animals were exposed to an ambient temperature of 34 ± 0.5°C (humidity 50–60 relative %) for 2 h ($n = 6$ for each group). Rectal temperature

was taken before the experiment and 1 and 2 h after the start of heat exposure.

All experiments were carried out between 8 a.m. and 3 p.m.

RESULTS

At the beginning of the test, all rats exhibited pronounced exploratory activity, and no apparent difference in behavior could be observed between the controls and the animals treated with DSP-4. However, the latency of escape and time spent on the platform were significantly different between the controls and the DSP-4-treated animals at all temperatures tested (Fig. 1A, B). The control animals spent approximately 50% of the time on the platform during the test period at 42°C, and the time spent on the platform increased significantly in parallel with the increase of the floor temperature. At a floor temperature of 42 or 44°C, animals treated with DSP-4 spent a considerably shorter time on the platform (about 20%), and the latency of escape was especially prolonged as compared with that for the control animals. At the higher temperatures (i.e., above the pain threshold), the escape latency of the treated animals was significantly shortened but still significantly longer than in the control animals.

In some experiments, the body temperature was measured before and after the heat escape test at a floor temperature of 48°C. Exposure of the rats to the test significantly elevated the body temperature (by about 1.5°C) in both groups ($p<0.001$, $n=8$ in each group). There was no significant difference in body temperature, however, between the two groups before (control: 36.6 ± 0.06°C; DSP-4-treated: 36.8 ± 0.09°C) or after (control: 38.3 ± 0.24°C; DSP-4-treated: 38.3 ± 0.08°C) completion of the test.

Similarly, no significant difference between DSP-4-treated and control rats was observed on exposure to an ambient temperature of 34°C for 2 h. The body temperatures in the control animals were 37.4 ± 0.13°C before and 39.6 ± 0.12 and 39.6 ± 0.07°C after 1 or 2 h of heat exposure, respectively; in DSP-4-treated rats, body temperatures were 37.6 ± 0.11°C before and 39.4 ± 0.09 and 39.4 ± 0.09°C after 1 or 2 h of heat exposure, respectively.

The heat pain sensitivity, as determined with the hot-plate method at 56°C, was the same in the controls (8.2 ± 1.24 s, $n=8$) and the DSP-4-treated animals (8.4 ± 1.83 s, $n=8$).

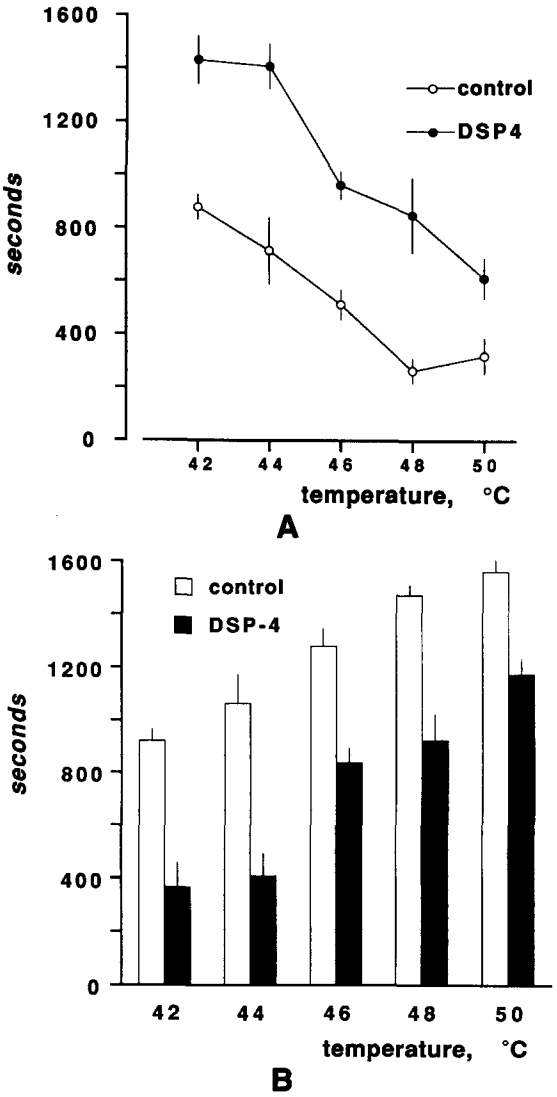

FIG. 1. Latency of escape reaction (A) and time spent on the platform (B) in control ($n=6$–8) and DSP-4-treated animals ($n=6$–8) at various floor temperatures. Bars indicate SEM. There was a significant difference between the control and DSP-4-treated rats at all tested temperatures ($p<0.01$; Student's t-test).

DISCUSSION

Systemic administration of DSP-4 is reported to produce a rapid and selective depletion of NA in the brain and spinal cord, and also in the peripheral adrenergic neurons (3, 11, 17). Whereas the peripheral reduction of NA is of a transient nature, the NA disappearance from the brain and spinal cord is long lasting (3,11) and most pronounced in the brain regions innervated by the LC neurons (17). Thus the selectivity for NA-containing neurons and the permanent effectiveness make DSP-4 a valuable tool for studies on the functional role of the LC noradrenergic neurons. In our studies, animals were subjected to a thermoregulatory behavioral test twelve days after DSP-4 treatment, i.e., when peripheral NA had returned to the physiological level, but no recovery could be observed in the LC-innervated regions of the central nervous system (11).

The results of the present study show that the depletion of NA from the central adrenergic neurons is associated with an imperfect heat defense behavior, similar to that observed previously after chemical lesioning of peripheral sensory C-afferents with capsaicin (13, 14, 16). This impairment was clearly apparent in the escape reaction from both nonnoxious and noxious heat stimuli, but the latency of the reaction was significantly shortened when the temperature was above the heat pain threshold.

During the heat escape, a significant increase in body temperature was observed, this being of the same magnitude in the controls and the DSP-4-treated animals. This slight hyperthermia could be a result of the increased ambient temperature, or it could be mediated emotionally by the stressful nature of the behavioral test. Since, similarly, there was no significant difference in the hyperthermia between the controls and the DSP-4-treated animals at high ambient temperature either, it can be concluded that DSP-4 treatment per se does not affect the autonomic thermoregulation. As mentioned above, DSP-4 mainly reduces the NA level in the brain regions innervated by the LC, thereby affecting NA-containing axons, e.g., in the cerebral cortex, while leaving the hypothalamic NA axons largely unaffected (17). Thus, even if NA neurons are essential in the autonomic control of body temperature, one would not expect an impaired

autonomic thermoregulation following DSP-4 treatment, since the NA innervation of the hypothalamus, the highest integrator of thermoregulation, is unaffected by the treatment (17). Taken together, these results indicate that DSP-4 treatment significantly impairs heat defense behavior, but this can be fully compensated by the autonomic regulation. In this regard, it is interesting to note that depletion of the central serotonin with p-chlorophenylalanine does not affect behavioral thermoregulation (16), although serotoninergic neurons are generally considered to be involved in thermoafferent signal transmission to the hypothalamus/preoptic region (7). Thus behavioral thermoregulation may be linked to the LC noradrenergic neurons, while the serotoninergic neurons may more specifically be involved in the autonomic control of body temperature.

The present results indicate a significant role of the central NA neurons in escape behavior. Depletion of NA with DSP-4 also causes a deficit in active avoidance tests involving electrical shock (2). These findings support the proposed function of LC in defense alarm reactions (6). LC neurons, however, are sensitive to both noxious heat and nonnoxious peripheral warm stimuli (4, 8, 9, 12). In the present experiment, the behavioral deficit was not specific to noxious heat: A form of behavioral thermoregulation, i.e., the escape reaction from a plate warmed to a temperature below the pain threshold, was also affected. It is concluded, therefore, that the defense alarm function of the noradrenergic neurons of LC is involved in the behavioral thermoregulation against a warm environment.

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