Effect of Substance P on Thermoregulation Parameters during Different Cooling Modes

E. Ya. Tkachenko, V. P. Kozaruk, and T. V. Kozyreva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 6, pp. 643-645, June, 2006
Original article submitted February 2, 2006

The modulatory effect of ionophoretic application of substance P to the skin on the formation of the cold-triggered thermal protection reactions depends on the rate of cooling. During rapid cooling substance P enhances heat production, while during slow cooling it promotes constriction of skin blood vessels aimed at reduction of heat emission.

Key Words: *thermal regulation; thermal receptors; cooling; substance P*

Neuropeptide substance P (SP) is located in sensory afferents of the same types as those supplying skin thermoreceptors. Cold-induced activation of the sympathoadrenal system can also mobilize SP, because sympathetic fibers contain not only norepinephrine, but also the neuropeptides, including SP [6,8]. We previously showed that injection of capsaicin promoting the release of SP and some other neuropeptides, modifies the threshold and amplitude of cold-protection reactions [2], which are the major regulated parameters in the thermoregulatory system.

In this paper, we examined the effect of ionophoretic application of SP to the skin on the threshold and amplitude of cold-induced thermoregulatory reactions. This mode of application provides maximum concentration of the agent in the zone of skin thermoreceptors.

MATERIALS AND METHODS

The experiments were carried out at room temperature (22°C) on male Wistar rats (body weight about 200 g, n=59) under nembutal narcosis (40 mg/kg). The rats were placed on a heated table (36°C). The abdominal skin (25 cm^2) was cooled with a thermode. Two modes of cooling chosen in previous electrophysiological studies [1] were used: rapid cooling (RC, 0.08°C/sec) and slow cooling (SC, 0.007°C/sec). In both cases cooling was continued until rectal temperature decreased by 3°C. Dynamic activity of cold receptors was revealed during RC, but not during SC.

In experimental rats, the examined skin area was subjected to SP ionophoresis immediately before cooling. Dissolved SP (0.1 mg/ml) was applied from the anode electrode (0.08 mA/cm) for 20 min. In control rats RC and SC were carried out without SP application. RC was applied to 13 control and 15 experimental rats and SC to 10 control and 13 experimental rats. A special series of experiments (n=10) showed that ionophoretic procedure *per se* had no effect on the examined parameters.

The recorded parameters were skin temperature in the auricle (distant site thermally isolated from the environment), total O_2 consumption, and electrical activity of cervical muscles. Monitoring of these parameters made it possible to assess vascular reaction characterizing heat emission to the environment and the metabolic response to cooling (shivering and non-shivering thermogenesis). The onset of thermoregulatory reaction was documented by a 0.1°C drop of skin auricular temperature during cooling, increased O_2 consumption (by 1 ml/min×kg), and increased electrical activity of the

Laboratory of Thermophysiology, Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** kozyreva@iph.ma.nsc.ru. T. V. Kozyreva

cervical muscles (by 1 μV). The maximum amplitude of the thermal reaction was determined at the end of cooling. The intracutaneous temperature of the cooled skin and rectal temperature were also measured. These parameters were used to assess cooling rate and the temperature thresholds of the cold-protection reactions. When skin or rectal temperature attained the threshold values, the protective cold reactions were activated.

The data were analyzed statistically using Student's *t* test and processed with original Term software.

RESULTS

Under thermally neutral conditions, ionophoretic application of SP produced no effect on the recorded parameters (Table 1).

Cooling induced systemic cold-protection reactions: decreased heat emission to the environment due to constriction of skin vessels and decrease in skin temperature and enhanced heat production due to increased oxygen consumption and shivering thermogenesis. The temperature thresholds and amplitude (maximum value) of the reactions differed depending on cooling rate and SP injection (Tables 2, 3).

SP had no effect on vascular reaction induced by RC. At the same time, SP decreased the temperature threshold of cold-induced metabolic response (as assessed by the drop of rectal temperature), while the amplitude of this reaction increased significantly relatively to the control group (Table 2). The temperature threshold of RC-triggered electrical response of cervical muscles was similar in the control and experimental groups, although the maximum value of muscle electrical activity was significantly grater in the experimental group. Therefore, during RC accompanied by transient activity of peripheral skin thermoreceptors the effects of SP manifested only in decreased threshold and increased amplitude of the metabolic response (*i.e.* enhanced heat production).

Increased metabolic response to RC after SP was predominantly related to enhanced muscle contractile activity. Ionophoresis creates high local concentration of the administered substance in the site of application, but direct or indirect systemic effects of SP cannot be excluded. In this case, changes in the parameters of thermal reactions (specifically, the temperature thresholds) can be explained by SP-induced activation of skin thermoreceptors, while up-regulation of metabolic response and shivering thermogenesis can be also caused by SP-induced increase of the blood flow and O_2 consumption in skeletal muscle and adipose tissue [7,9].

In SP-treated rats, SC was associated with a decrease in the threshold of vascular reaction, while the thresholds and amplitude of the metabolic response and muscle electrical activity remained unchanged. Peptidergic nerve fibers are involved in local regulation of blood flow, but the effect of tachykinins (*e.g.* SP) is unstable and can result in both vasodilation (against the background of high tone *e.g.* after nonepinephrine treatment and vasoconstriction, although at low vascular tone) [9]. Probably, potentiation of vasoconstriction during SC in rats receiving SP and the absence of the modulatory effect of this peptide on vascular reactions induced by RC can be explained by differences in the degree of sympathetic activation during various cooling rates [5].

We previously showed that RC and SC after ionophoretic application of capsaicin to the cooled skin were accompanied by activation of heat-protection and inhibition of cold-protection reactions together with an increase of their thresholds [2]. Comparison of modulatory effects of capsaicin and SP on the thresholds of cold-protection reactions attests to different modes of action of these substances and suggests that the effect of SP on the cold-protection reactions is not mediated via the vanilloid receptors.

Therefore, the character of modulatory effect of SP on the thresholds and amplitudes of the cold-protection reactions depends on the cooling rate and consequently, on the presence or absence of the dynamic (transient) component of the thermoreceptor activity. Our previous studies showed that dynamic activity of peripheral thermoreceptors plays

TABLE 1. Effect of SP on Thermal Protection Reaction under Thermoneutral Conditions (*M*±*m*)

Parameter	Norm	Control	Experiment (SP)
Total O_2 consumption, ml/min×kg	19.50±0.60	18.9±0.89	18.70±0.59
Temperature of auricular skin, °C	30.00±0.251	30.4±0.51	29.30±0.48
Temperature of abdominal skin, °C	38.50±0.30	38.3±0.29	38.00±0.17
Rectal temperature, °C	37.70±0.13	37.5±0.21	37.30±0.17
Electrical activity of cervical muscle, μV	3.50±0.27	3.4±0.31	3.10±0.35

TABLE 2. Effect of SP on Temperature Thresholds and Amplitude of Thermoregulatory Reactions Induced by RC (*M*±*m*)

Parameter		Control	SP
Vascular reaction	skin temperature threshold shift, °C	3.30±0.54	3.50±0.71
	amplitude, °C	2.90±0.24	3.30±0.83
Metabolic response	skin temperature threshold shift, °C	10.30±0.99	10.60±0.58
	rectal temperature threshold shift, °C	2.70±0.65	1.00±0.07*
	amplitude, ml/min×kg	34.7±2.1	56.90±4.97*
Muscle shivering activity	skin temperature threshold shift, °C	9.00±0.51	9.40±1.43
	rectal temperature threshold shift, °C	1.10±0.57	0.80±0.25
	amplitude, μV	14.60±2.54	22.40±1.64*

Note. Here and in Table 3: *p<0.05 compared to the control.

TABLE 3. Effect of SP on Temperature Thresholds and Amplitude of Thermoregulatory Reactions Induced by SC (*M*±*m*)

Parameter		Control	SP
Vascular reaction	skin temperature threshold shift, °C	3.10±0.66	1.80±0.47*
	amplitude, °C	2.60±0.41	2.70±0.38
Metabolic response	skin temperature threshold shift, °C	8.50±0.71	6.80±0.47
	rectal temperature threshold shift, °C	2.50±0.23	2.30±027
	amplitude, ml/min×kg	38.40±3.83	40.90±3.87
Muscle shivering activity	skin temperature threshold shift, °C	7.10±0.98	7.10±0.68
	rectal temperature threshold shift, °C	1.90±0.23	2.50±0.28
	amplitude, μV	13.40±2.95	20.80±2.45

an important role in the regulation of functional systems, maintaining temperature homeostasis and in modulation of the hormonal status of the organism during cooling. It can be hypothesized that different degree of participation of sympathetic system during various modes of cooling [3-5] can be one of the reasons underlying different effects of SP observed during RC and SC.

REFERENCES

1. T. V. Kozyreva and L. A. Verkhoglyad, *Ross. Fiziol. Zh.*, **83**, Nos. 11-12, 135-142 (1997).
2. T. V. Kozyreva, E. Ya. Tkachenko, and V. P. Kozaruk, *Byull. Sib. Otdel. Ross. Akad. Med. Nauk*, **No. 1**, 119-122 (2002).
3. T. V. Kozyreva, E. Ya. Tkachenko, and V. P. Kozaruk, in: *Ergonomics*, New York (1998), pp. 137-141.
4. T. V. Kozyreva, E. Ya. Tkachenko, and V. P. Kozaruk, *J. Therm. Biol.*, **24,** 175-183 (1999).
5. T. V. Kozyreva, E. Ya. Tkachenko, V. P. Kozaruk, *et al., Am. J. Physiol*., **276,** No. 6, Pt. 2, R1668-R1672 (1999).
6. S. Matsushima, Y. Sakai, and Y. Hira, *Microsc. Res. Tech.*, **46**, Nos. 4-5, 265-280 (1999).
7. T. Osaka, A. Kobayashi, Y. Namba, *et al., Pflugers Arch.*, **437**, No. 1, 36-42 (1998).
8. S. Reuss, *Microsc. Res. Tech.*, **46**, Nos. 4-5, 305-309 (1999).
9. C. Severini, G. Improta, G. Falconieri-Erspamer, *et al., Pharm. Rev.*, **54**, 285-322 (2002).