Effects of Hypothermia on Norepinephrine Release and Effector Response in Isolated Perfused Cat Spleen

By Åke Wennmalm, Per Hedqvist, and Lennart Stjärne

ABSTRACT
The effects of hypothermia on the sympathetic neuroeffector junction were studied in the isolated perfused cat spleen. The experiments were aimed at determining the temperature dependence both of prejunctional events, i.e., efflux of norepinephrine from the sympathetic nerves in response to electrical stimulation or to indirectly acting sympathomimetic amines like tyramine or phenethylamine, and of postjunctional events, i.e., the contractile response of the smooth muscle to the norepinephrine liberated. Hypothermia was found to depress both the norepinephrine efflux in response to stimulation and the contractile response to the endogenous norepinephrine released, as well as to exogenous norepinephrine. However, these processes were found to respond differently to temperature change. Thus hypothermia depressed norepinephrine release as well as the pressor responses to tyramine and phenethylamine much more strongly than the corresponding responses to electrical nerve stimulation or the pressor response to exogenous norepinephrine. The results strongly support the concept that sympathomimetic amines release norepinephrine from sympathetic nerves by mechanisms which differ from those mediating norepinephrine release induced by depolarization.

ADDITIONAL KEY WORDS sympathetic nerves nerve stimulation tyramine temperature smooth muscle

The sympathetic nervous system plays an important role in thermal homeostasis during general exposure to cold, which induces increased activity in the sympathetic nerves, as reflected by a marked increase in the urinary excretion of norepinephrine (NE) (1). However, less is known concerning the direct local effect of hypothermia on sympathetic nerve function, such as processes involved in conduction of impulses in C fibers, neurotransmitter release, and sensitivity of the smooth muscle to the transmitter released.

Nerve conduction is known to be temperature dependent. Thus, Franz and Iigo (2) found that in nonmyelinated nerve fibers there was a progressive reduction in conduction velocity as the temperature was lowered from 37°C down to 8°C, at 10°C being reduced to 15 to 20% of the 37°C value.

The effect of hypothermia on neurotransmitter release has so far not been extensively investigated. In studies of the release of NE from the sympathetic nerves of the isolated cat spleen Kirpekar et al. (3) found that reduction of the perfusion temperature to 15°C markedly reduced the amount of NE released in response to nerve stimulation or to infusion of potassium. However, the authors did not present quantitative data concerning the extent of reduction of NE release during hypothermia.

The spontaneous release rate of NE from isolated bovine splenic nerve granules was found to be reduced to about 10 percent when the temperature of the incubation medium was reduced from 37°C to 20°C (4, 5). However, the significance of the spontaneous release of NE from isolated granules with
respect to the release of NE from the nerves in response to depolarization is not known.

The sensitivity of smooth muscle to catecholamines has been reported to be altered by low temperature. Thus Smith (6) stated that vessels stimulated by epinephrine contracted and relaxed more slowly at 17°C than at 37°C. Keatinge (7) found that the response of isolated vascular strips to epinephrine progressively decreased when the temperature was lowered from 36°C, being completely abolished between 12.5°C and 10°C. In using a similar preparation, Sams and Winkelmann found a decrease in catecholamine-induced contraction and in addition a changed response pattern when the temperature was lowered (8). On the other hand, hypothermia has been reported to potentiate certain smooth-muscle effects of catecholamines. Thus, the cutaneous veins of dogs contract more strongly in response to NE when the temperature is lowered from 37°C down to 17°C (9). In addition, epinephrine has been shown to inhibit the spontaneous activity of the isolated rabbit small intestine more effectively at 30°C than at 38°C (10). An increased response during hypothermia has also been found in the isolated guinea pig vas deferens by Della Bella et al. (11). They found that the contraction on nerve stimulation increased 5 to 10 times when the temperature was lowered from 32°C to 20°C.

**Methods**

Cats of both sexes weighing 2.5 to 4.5 kg were used for the study. The experimental animals were anesthetized with sodium pentobarbital (30 mg/kg ip) and heparin (1000 IU/kg iv). The abdomen was opened by a midline incision and the spleen together with its vascular and nervous supply was isolated. The organ was transferred to a perfusion chamber and perfused with a physiological salt solution (in mM: NaCl, 103.4; KCl, 4.8; MgSO₄ · 7 H₂O, 1.2; CaCl₂, 2.5; Na-pyruvate, 4.8; Na-1-glutamate 2.2; KH₂PO₄, 1.0; Na HCO₃, 25; dextrose, 11.5; ascorbic acid, 0.11) aerated with 5% CO₂ in O₂. The flow rate was kept at 7.5 ml/min by a constant rate perfusion pump. Perfusion pressure was registered with a Statham pressure transducer connected to a Grass Model 7 polygraph. The effluent from the spleen was divided into 10 ml portions by means of an automatic fraction collector. Supramaximal electrical stimulation of the splenic nerves was carried out with platinum electrodes, using a Grass S4 stimulator, operated at a frequency of 10 cps, a duration of 2 msec, and a stimulation strength of 10 to 15 volts, for stimulation periods of 20 seconds, separated by an interval of 10 minutes. The temperature was measured in the perfusing medium, in the perfusion chamber, and on the surface of the spleen.

The experiment was started by an intra-arterial infusion of 30 μg of tritium-labeled dl-NE (specific activity 5 mc/μmole, New England Nuclear Corp.). After washing for 30 minutes by perfusion with NE-free Krebs-Henseleit's solution, the spleen was exposed either to nerve stimulation or to intra-arterial injection of NE (0.3 to 0.4 μg), phenethylamine (20 μg) or tyramine (20 μg). The temperature of the system was varied stepwise between 8°C and 37°C, both increasing and decreasing the temperature. The nerve stimulation or injection of NE, phenethylamine, or tyramine was carried out at each step.

The time course and profile of the NE output from the sympathetic nerves in the cat spleen was monitored by measuring the amount of radioactivity in each 10-ml fraction of the effluent from the perfused organ. The radioactivity of the different samples was determined by counting 0.5-ml aliquots in a Packard Liquid Spectrometer using a 7:3 toluene-absolute ethanol solution containing 4 g of 2,5-diphenyloxazole and 100 mg of 1-4-bis-(4-methyl-5-phenyloxazoly) benzene per liter of toluene. Quenching was monitored by internal standards of ³H-dl-NE.

**Results**

According to previous evidence, almost all of the radioactive material released in response to nerve stimulation or to injection of tyramine in the preparation used consists of intact NE (12). Reduction of the temperature of the spleen depressed the efflux of NE and the pressor effects caused by electrical nerve stimulation as well as by phenethylamine or tyramine (Figs. 1, 2). However, the tyramine-induced overflow of NE was clearly more temperature dependent (Fig. 2). Similar results were obtained with phenethylamine. The relative fall in the NE-releasing effect of tyramine was significantly more marked than that of electrical nerve stimulation, both at 25°C and 15°C.

The average resting perfusion pressure was...
SYMPATHETIC NERVE FUNCTION IN HYPOTHERMIA

Typical experiment showing the effect of hypothermia on outflow of radioactivity and pressor response to 20 seconds of nerve stimulation in the isolated cat spleen.

Effect of hypothermia on the outflow of radioactivity from isolated cat spleens in response to nerve stimulation or tyramine injections. Vertical bars = means ± se (n = 5) in percent of control values obtained at 37°C. Nerve stimulation and tyramine injection values statistically separated at 25°C (P < 0.01) and at 15°C (P < 0.02). TA = tyramine.

about 25 mm Hg. During hypothermia this pressure rose to 30 mm Hg in some of the experiments. At 37°C the pressor response to nerve stimulation was 20 to 30 mm Hg. This
response progressively decreased with the fall in temperature (Fig. 3) to be almost completely abolished at temperatures below 15°C. Virtually the same effect was obtained when nerve stimulation was replaced by injection of constant amounts of NE (Fig. 4). The pressor responses to tyramine and phenethylamine were about 5 mm Hg at 37°C in all experiments. This response rapidly decreased when the temperature was lowered, and was completely lost at around 25°C.

Discussion

Hypothermia was found to depress the NE efflux and the pressor responses both to depolarization and to tyramine or phenethylamine, although to a different extent. The reduced NE overflow on nerve stimulation might be due to direct effects of hypothermia on nerve conduction, on release of NE from the nerves, or on removal or enzymatic inactivation of the NE released. Nerve conduction is blocked between 1° and 7.1°C (2, 13). Thus, impaired nerve conduction might have influenced the overflow of NE to some extent in the lower temperature range of the present study. However, at moderate hypothermia impaired conduction was probably not of any major importance.

Release of NE from the nerves in response to nerve stimulation has previously been shown to be depressed by hypothermia (3). This is in accordance with the present results. The process of spontaneous release of NE from isolated bovine splenic nerve granules is highly temperature dependent. The basic release rate at 37°C is 8 to 10 times higher than that at 20°C (4, 5). However, the outflow of NE in response to nerve stimulation in the present series was far less temperature dependent, the ratio of NE efflux at 37°C and that at 20°C being about 1.5. Thus it seems unlikely that the release of NE from the nerves in response to depolarization is immediately dependent on the same mechanisms as those determining spontaneous release of NE from isolated nerve granules in the temperature range mentioned. Reduction of temperature below 20°C produced a pronounced depression of the overflow of NE in response to nerve stimulation. During such extreme hypothermia the temperature dependence of the outflow response to nerve stimulation was close to that found for tyramine and phenethyl-
lamine in the 37° to 25°C temperature range.

On the other hand, the temperature dependence of the phenethylamine- or tyramine-induced release of NE from the nerves was more closely related to that of spontaneous release of NE from isolated nerve granules. This may be of particular significance since the NE-releasing effect of tyramine on the nerves appears to be exerted in part by direct action on the granules (14). At this level tyramine may act both by displacement of granule-bound NE (15) and by inhibition of reuptake into the granules of the NE released, spontaneously or by tyramine. Thus the observed similarity in temperature dependence between spontaneous release of NE from isolated granules in vitro and tyramine-induced release of NE from the whole neuron, may indicate that spontaneous release of NE from granules in situ may be rate limiting for the effect of tyramine on the nerves. Hypothermia may in addition interfere with the effects of tyramine at other levels, such as on the uptake of tyramine into the nerves (12) and on the tyramine-induced inhibition of reuptake into nerves of the NE released (16, 17).

The vasoconstrictor effect of adrenaline has been shown to be reduced by low temperature (6, 7). However, it has recently been shown that the response to NE is markedly increased in dog's cutaneous veins (9). In the present study the pressor effect in response to nerve stimulation gradually decreased when the temperature was lowered. Not only was the amplitude of the response altered, but also the pattern of the curve. At 37°C there was a sharp initial rise at the beginning of the nerve stimulation or NE injection, but during hypothermia this sharp rise became gradually less pronounced, giving the curve a more flattened profile.

In conclusion, hypothermia depresses several functions of the sympathetic neuroeffector system of the isolated perfused cat spleen, apparently by reducing NE release from the nerves as well as the sensitivity of the smooth muscle to NE. However, there is a marked difference in the influence of hypothermia on the effect of the two stimuli used for inducing NE release, in the 37° to 20°C temperature range. Thus, the "physiological" release of NE induced by nerve impulses is much more resistant to hypothermia than the "pharmacological" release induced by sympathomimetic amines. This supports the concept that these two types of NE release depend on distinctly different mechanisms. It also indicates that "physiological" release, i.e., secretion on depolarization, is not immediately dependent on spontaneous release of NE from nerve granules, while "pharmacological" release may well be so (cf. 18).

References


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