Effect of Splanchnic Nerve Stimulation on Epinephrine and Norepinephrine Release from Gastrointestinal Sympathetic Postganglionic Endings in the Frog

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Summary Gastrointestinal vascular perfusates in bullfrogs before and after splanchnic nerve stimulation were collected and analyzed for epinephrine and norepinephrine. Catecholamine in the perfusate before stimulation was predominantly epinephrine. Upon stimulation a marked increase in epinephrine release without any significant change in norepinephrine release was observed in experiments at 8–10°C as well as 16–21°C, showing that in frogs epinephrine is the sympathetic neurotransmitter and that the release of epinephrine is not affected by low temperatures.

Key words : frog, splanchnic nerve, epinephrine release.

The release of norepinephrine from sympathetic nerve terminals in warm-blooded animals has been clearly established [1]. In the frog, however, it has been reported that the adrenergic transmitter found in heart extracts is predominantly epinephrine [2, 3] and that epinephrine content is not affected by decreases in environmental temperatures [3]. Experiments on the toad using perfused blood vessels of the stomach and intestines showed that splanchnic nerve stimulation induced a release of a vasoconstrictor substance [4, 5], which was proved to be an epinephrine-like substance [6]. However, no differential estimations for epinephrine or norepinephrine were made in those experiments. It remains unclear, therefore, whether the neurotransmitter released from splanchnic nerve terminals in this animal species is epinephrine or norepinephrine.

The purpose of this study was to determine the effect of splanchnic nerve stimulation on epinephrine and norepinephrine release from sympathetic postganglionic endings and to study the influence of low temperatures on the catecholamine release in the frog.

Bullfrogs (*Rana catesbeiana*) of either sex were divided into the following two

groups: 1) the spring group had six bullfrogs weighing 380–426 g each who were studied at 16–21°C; 2) the winter group had eight bullfrogs weighing 200–272 g each studied at 8–10°C. Gastrointestinal vascular perfusion was performed with a slight modification of the method by Koono [5]. Animals were decapitated and pithed. The right aorta, splenic artery and vein, both renal arteries and veins, and the abdominal aorta at a point just inferior to the celiaco-mesenteric artery were ligated. The right splanchnic nerves were then cut. The perfusion medium was Ringer solution (pH 7.2) containing 119.7 mM NaCl, 1.6 mM KCl, 1.2 mM CaCl_2 , and 3.8 mM NaHCO_3 . A polyethylene tube for infusing the Ringer solution was inserted into the left aorta, and perfusion pressure was maintained at approximately 42 cmH₂O. Another polyethylene tube was inserted into the portal vein to collect the perfusate. The left splanchnic nerves were then ligated and cut, and the peripheral ends of the nerves were stimulated for 2 min (1.5 V, 30 Hz, 1 ms duration). The perfusate flowing from the portal vein was collected for 2 min prior to stimulation. Immediately after the start of stimulation, the perfusate was collected in five consecutive 2-min intervals. Perfusates collected at 2-min intervals before, during, and after stimulation amounted to 0.7–1.8, 0.3–1.5, and 0.3–2.2 ml, respectively. The total volume of each 2-min perfusate was used for catecholamine assay.

The perfusate was analyzed for epinephrine and norepinephrine by high performance liquid chromatography with electrochemical detection [7]. Perfusate concentrations of epinephrine and norepinephrine were corrected for loss during extraction using the recovery of a dihydroxybenzylamine internal standard. Intra-assay coefficients of variation determined from eight repeated determinations of a control perfusate were 8.2% for epinephrine (600 pg/ml) and 7.1% for norepinephrine (1,000 pg/ml). The detection limit was 20 pg for each catecholamine. All data are presented as means $\pm$ SEM. Statistical analysis was performed using Student's *t*-test.

Results are shown in Fig. 1. Taking the large differences in body weight between the winter and spring groups into consideration, the rate of releases of epinephrine and norepinephrine was expressed as pg per g body weight per min. Before stimulation the predominant catecholamine in gastrointestinal vascular perfusate was epinephrine. The release of epinephrine was 0.86 ± 0.13 pg/g/min in the spring group and 1.04 ± 0.06 pg/g/min in the winter group. The percentages of epinephrine in total catecholamine (epinephrine+norepinephrine) in the spring and winter groups were 87.3 ± 6.2 and $91.1 \pm 0.3\%$, respectively. Stimulation of the splanchnic nerves resulted in a marked increase in epinephrine release ($p < 0.01$) without any significant change in norepinephrine release ($p > 0.05$) in both groups. The maximal release of epinephrine occurred at 2–4 min after the onset of stimulation, at which time the percentages of epinephrine in the spring and winter groups were 95.8 ± 1.0 and $89.3 \pm 3.6\%$, respectively. Then epinephrine release decreased slowly and returned to the level before stimulation by 8–10 min. The rate of releases of epinephrine and norepinephrine as well as the percentage of epinephrine

Fig. 1. Epinephrine and norepinephrine release from sympathetic postganglionic endings in bullfrogs before and after splanchnic nerve stimulation. Spring group: ○, epinephrine; ●, norepinephrine. Winter group: △, epinephrine; ▲, norepinephrine. Horizontal solid bar, splanchnic nerve stimulation. Data are expressed as means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, compared with the rate of release before stimulation.

in the total catecholamines during each period in the winter group were not significantly different from those in the spring group ($p > 0.05$).

It was shown that a vasoconstrictor substance [4, 5] or an epinephrine-like substance [6] was released into gastrointestinal vascular perfusates following splanchnic nerve stimulation in toads. In those experiments, the perfusate was analyzed for this substance using the toad sino-atrial preparation method, by which epinephrine and norepinephrine could not be differentially estimated. In the present study, catecholamine released from splanchnic postganglionic endings in bullfrogs was evaluated by differential estimations for epinephrine and norepinephrine. A marked increase in epinephrine release without any significant change in norepinephrine release following splanchnic nerve stimulation was observed, indicating that in frogs epinephrine is the sympathetic neurotransmitter. By contrast, however, splanchnic nerve stimulation experiments on dogs with gastrointestinal vascular perfusion by Malmjac *et al.* [8] showed that the predominant sympathetic postganglionic neurotransmitter in mammalian species was norepinephrine.

As to the influence of environmental temperatures on the catecholamine content in tissues which have sympathetic innervation, there is evidence that the epinephrine content of the heart of frogs kept at a low temperature for 1 week is not significantly different from that of frogs kept at room temperature [3]. Such a finding is consistent with the results of this study which showed no significant difference in the amounts of epinephrine in gastrointestinal vascular perfusates between the winter and spring groups.

In bullfrog heart perfusion experiments, Azuma *et al.* [3] reported that sympathetic stimulation produced a significant increase in epinephrine release with very small amounts of norepinephrine release. The present observation together with the finding by Azuma *et al.* [3] suggests that in the frog the sympathetic control of the heart and the blood vessels of the stomach and intestines is mediated by epinephrine, but not by norepinephrine.

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