Suppression of Clonidine-Induced Vasoconstriction by Cooling

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Abstract—Using the cannula inserting method, we investigated whether vascular responses to norepinephrine, phenylephrine, clonidine, tyramine and KCl were altered by cooling (37°C to 27°C) in isolated canine ear arteries. Vasoconstrictor responses to norepinephrine, phenylephrine and tyramine were slightly depressed or unchanged, whereas those to clonidine and KCl were significantly suppressed by cooling. It is suggested that activation of Ca channels via alpha-2 adrenoceptors may be depressed by cooling in dog ear arteries.

Recently, Ito and Chiba (1) found that not only alpha-1 but also alpha-2 adrenoceptors are involved in the vasoconstrictor responses of the isolated and perfused canine intermediate auricular artery (ear artery) by using a cannula inserting technique (2, 3).

Patton and Wallace (4) reported that moderate cooling potentiated responses to norepinephrine and epinephrine of isolated rabbit ear arteries. In 1985, Flavahan et al. (5) reported that cooling (from 37°C to 24°C) augmented contractions to norepinephrine and the alpha-2 adrenergic agonists B-HT920 and UK14304, but did not affect responses to the full alpha-1 adrenergic agonist phenylephrine in isolated canine cutaneous veins. On the contrary, Harker and Vanhoutte (6) reported that cooling did not significantly modify contractile responses to norepinephrine in the central ear artery of the rabbit.

In the present study, experiments were designed to determine the effects of cooling (from 37°C to 27°C) on responses to alpha-1 and alpha-2 adrenergic activations in isolated canine ear arteries which are rich in alpha-1 and alpha-2 adrenoceptors as reported previously (1).

Mongrel dogs of either sex (5–17 kg) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After treatment with sodium heparin (200 units/kg, i.v.), the animals were sacrificed by rapid exsanguination from the right common carotid artery. The intermediate auricular (ear) artery of either ear was then carefully isolated, and 1–3 segments (without large branches, 0.4–0.7 mm in outer diameter, 4–7 mm in length) were cut from each isolated artery. The segments were cannulated and set up for perfusion with a constant flow rate. The perfusate contained 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgCl2, 2.5 mM CaCl2, 25 mM NaHCO3 and 10 mM glucose and was bubbled with a mixture of 95% O2 and 5% CO2 and maintained at a constant temperature of 37°C. The rate (1–2 ml/min) was adjusted at the beginning of the experiments to obtain a control perfusion pressure of approximately 100 mmHg. The perfusion pressure was measured with an electronic manometer, and the vasoconstriction induced by intraluminal administration of a drug was recorded as an increase in perfusion pressure. Drugs used were dl-norepinephrine hydrochloride (Sankyo), phenylephrine (Wako), clonidine hydrochloride (Boehringer Ingelheim), tyramine hydrochloride (Tokyo Kasei) and potassium chloride. The drugs were dissolved in saline. Each drug solution was intraluminally administered into the perfusion line close to the cannula in a volume of 0.01–0.03 ml over 4 sec by use of a microinjector (Terumo Co.). The data are shown as means±S.E.M. Student’s t-test was used, and a P value of 0.05 or less was considered significant. The experiments were started when the arteries had equilibrated for about 1–2 hr, until perfusion pressure became stable.
Norepinephrine, phenylephrine and clonidine induced a vasoconstriction in a dose-related manner when injected into cannulated arterial preparations at 37°C as reported previously (1). When the temperature of the perfusion circuit including the organ was changed from 37°C to 27°C within 30 min, basal perfusion pressure was increased approximately 10 mmHg, and stable perfusion pressure was maintained during the experiment. At 27°C, vasoconstrictor responses to norepinephrine and phenylephrine were slightly depressed. However, clonidine induced vasoconstrictions were depressed in doses of 0.3 and 1 µg by cooling as shown in Fig. 1. An indirect sympathomimetic amine, tyramine usually caused marked vasoconstrictions in canine ear arteries (7). Tyramine-induced vasoconstrictions were not significantly different between responses at 27°C and 37°C as shown in Fig. 2. A non-adrenergic vasoconstrictor substance, KCl, induced a relatively small vasoconstriction in these preparations. The KCl-induced vasoconstrictions at 37°C were significantly depressed by cooling to 27°C as shown in Fig. 2.

Flavahan et al. (5) reported that moderate cooling increased the contractile responses of the canine saphenous vein to norepinephrine under control conditions or following alpha-1 adrenoceptor blockade with prazosin, but not following alpha-2 adrenoceptor blockade with rauwolscine. Thus, they suggested that it is only the alpha-2 adrenergic component of the response to norepinephrine that is increased by cooling. Moreover, they demonstrated that cooling augmented the contractile responses evoked by low concentrations of the alpha-2 adrenoceptor agonists UK14,304 and B-HT920. On the other hand, Harker and Vanhoutte (6) reported that in the central ear artery of the rabbit, cooling appears to have very little effect on adrenergically induced contractions. Based on their experiments using the selective alpha-2 adrenoergic agents UK14,304 and rauwolscine, no evidence was obtained, suggesting a lack of an important role for alpha-2 adrenoceptors.
in the central artery of the rabbit ear. They considered that the relative absence of the alpha-2 adrenoceptor subtype in the central ear artery of the rabbit correlates with the lack of a potentiating effect of cooling on adrenergically induced contractions.

In the present study, we used isolated canine intermediate auricular (ear) arteries in which exist alpha-2 adrenoceptors (1). We demonstrated that cooling did not potentiate but significantly depressed vasoconstrictor responses to an alpha-2 adrenoceptor agonist, clonidine, in canine ear arteries. Recently, Ito and Chiba (8) reported that in isolated dog ear arteries, calcium antagonists similarly suppressed either clonidine- or phenylephrine-induced vasoconstrictions with relatively small, but a relatively large dose of calcium antagonist depressed clonidine-induced responses more than phenylephrine-induced ones. Cooling may cause a decrease in Ca influx from the extracellular space by activation of alpha-2 adrenoceptors, because the KCl-induced vasoconstrictions that might be produced by Ca influx were also significantly suppressed by cooling in this study, suggesting depression of Ca influx by cooling.

Another point of interest in the present study is that tyramine-induced constrictions were little or only minimally affected by cooling, although KCl-induced constrictions were markedly depressed. It was reported that the rate of vascular uptake of catecholamines was temperature-dependent (9). Concerning a release of catecholamines from nerve terminals by tyramine, Ca ions may not have an important role in perfusion experiments in these temperature ranges. It will be necessary to examine other catecholamine releasers and uptake blockers in perfusing preparations in order to elucidate the mechanism of tyramine action.

In the present study, we used clonidine as an alpha-2 adrenoceptor agonist. However, it has been also reported that clonidine is a relatively selective alpha-2 agonist because it contains not only alpha-2 but also alpha-1 adrenoceptor stimulating properties (10). Thus, it will be necessary to examine other selective alpha-2 adrenoceptor stimulants and antagonists in the future. Moreover, species differences and regionally different vessels may induce different results because of morphological differences or specific receptor densities.

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