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DIFFERENTIAL EFFECTS OF ACETYLCHOLINE ON CORONARY FLOW IN ISOLATED HYPOTHERMIC HEARTS FROM RATS AND GROUND SQUIRRELS

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Summary

This study was designed to determine whether cholinergic receptors are operative in the coronary vessels of a hibernating species (golden mantled ground squirrel, *Spermophilus lateralis*) and a nonhibernating species (rat, *Rattus norvegicus*) under normothermic and hypothermic conditions. Coronary flow and left ventricular systolic pressure were measured in isolated perfused hearts from squirrels at 37, 20 or 7˚C and from rats at 37 and 20˚C. During cooling, rat hearts became arrhythmic and failed between 15 and 12˚C. Squirrel hearts remained functional at 7˚C. Bolus injections of acetylcholine (>1.0 μg) caused significant coronary vasoconstriction in rat hearts at 37 and 20˚C. Similar treatment caused mild coronary vasodilation in squirrel hearts at both temperatures. Squirrel hearts did not respond to acetylcholine at 7˚C. The responses in both species were blocked by atropine. Rat coronary vessels appear to contain muscarinic constrictor receptors similar to those described in humans, sheep, cattle and pigs. The coronary vessels of squirrels, by contrast, do not. In this latter species there appears to be a preponderance of muscarinic (possibly endothelial-relaxing-factor-linked) dilator receptors. Given that acetylcholine acts only as a mild vasodilator at higher temperatures in squirrels, parasympathetic regulation of coronary flow in the squirrel heart is unlikely, especially during hibernation.

Introduction

The cardiovascular system of hibernating mammals is adapted to function at relatively low temperatures (body temperature, T_b 1–7˚C). Upon entrance into hibernation, heart rate and cardiac output fall along with metabolic rate. Although stroke volume and peripheral resistance increase, mean arterial pressure decreases (Lyman, 1982; Burlington and Darvish, 1988). The hearts from nonhibernating mammals become arrhythmic and/or fibrillate and cease to function between 10 and 15˚C, but the hearts of

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hibernating mammals are resistant to fibrillation or cardiac arrest and continue to function at temperatures approaching 1˚C (Lyman, 1982). Studies of isolated hearts suggest that one factor contributing to this difference may be that coronary flow and its autoregulation are maintained at low temperatures in hibernating, but not in nonhibernating, mammals (Burlington *et al.* 1989). How this is achieved in hibernating species is not clear. At low temperatures, *in vitro* coronary flow is independent of oxygen consumption and not entirely dependent on perfusion pressure (Burlington *et al.* 1989). Therefore, the intrinsic mechanism(s) involved in flow regulation remains unidentified. It is also not clear whether there are differences between hibernating and nonhibernating species in terms of extrinsic regulation of coronary flow. Indeed, the exact metabolic or neural control mechanisms which regulate coronary flow in any species are still being debated (Feigl, 1983; Miller, 1991). Recent evidence, however, suggests that flow may be partially regulated by cholinergic constrictor receptors in the coronary vessels and that coronary constriction, rather than dilation, may be the primary response to acetylcholine in most non-hibernating species (Kalsner, 1989). The present study was designed to assess whether cholinergic receptors are operative in the coronary vessels of hibernating and nonhibernating mammals under normothermic and hypothermic conditions. This was achieved by administering varying doses of acetylcholine to normothermic and hypothermic hearts isolated from a hibernator, the golden mantled ground squirrel [*Spermophilus lateralis* (Say)], and a nonhibernator, the laboratory rat (*Rattus norvegicus*).

Materials and methods

Adult ground squirrels and rats were housed at 23±2˚C with a 12h:12h L:D photoperiod. During November, December and January, a second group of squirrels was housed at 5±2˚C with a 2h:22h L:D photoperiod. These animals were provided with nesting materials and they entered hibernation readily (T_b, 5–8˚C). All animals were provided with Purina Laboratory Chow and water *ad libitum*. Ground squirrel diets were supplemented with fresh fruit, vegetables and sunflower seeds.

Each animal received 500i.u. of heparin intraperitoneally 1h prior to cervical dislocation. Thereafter, hearts were rapidly excised and placed in Krebs–Henseleit bicarbonate buffer (KHB) containing (in $mmol\,l^{-1}$): NaCl, 118; KCl, 4.7; KH_2PO_4, 1.2; $CaCl_2{\cdot}H_2O$, 3; $NaHCO_3$, 25; $MgSO_4$, 1.2; glucose, 11. The solution was gassed with 95% O_2 and 5% CO_2, adjusted to pH7.4 and maintained at less than 10˚C.

The aortic root of the isolated heart was attached to a stainless-steel perfusion cannula with 4-0 silk thread. The heart was then perfused with KHB solution maintained at the temperature (37, 20 or 7˚C) selected for that experiment. A modified Langendorff apparatus was used to measure left ventricular systolic pressure (V_{LP}) (Burlington *et al.* 1989). Perfusion pressure was maintained at 12kPa (90mmHg). A compliant latex balloon (Young Rubber) was connected to a 10cm section of polyethylene tubing (Intramedic, PE-100), inserted into the left ventricle *via* the left atrium, and secured with 4-0 silk. The tubing was connected to a Gould Statham P23 ID pressure transducer. A 500 μl syringe was attached to the tubing by a TC-18/3 three-way connector (Small

Parts). This system was used to maintain end-diastolic pressure at 1.3kPa (10mmHg). The balloon, tubing, syringe and transducer dome were filled with bubble-free deionized water. The transducer was connected to a Grass 7P1A preamplifier, a 7DAC amplifier and a 7WC8PA oscillograph.

Bipolar platinum electrodes were placed on the surface of the right atrium and a Grass stimulator was used to pace the hearts at 300beatsmin^{-1} at 37˚C, 72beatsmin^{-1} at 20˚C or 18beatsmin^{-1} at 7˚C. A separate series of experiments was conducted with spontaneously beating hearts. Coronary flow (f_C) was measured with a calibrated Gilmont flowmeter and checked by collecting exuded heart perfusate in a 25ml graduated cylinder. The temperature of the perfusate was controlled by a refrigerated water bath, and heart temperature was monitored with a BAT 12 Sensortex needle thermometer.

A 1ml syringe was initially used to inject saline or saline+acetylcholine into the tubing above the aortic cannula. Injection of as little as 0.1ml of saline alone caused significant transient changes in f_C and systolic pressure development. This problem was alleviated by injecting the solutions in bolus microlitre quantities using a 1 μl syringe.

f_C was measured after a 20min control period. We observed the effects on f_C of randomly ordered doses of acetylcholine (ACh) (0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 5.0, 10.0 or 20.0 μl in saline). Each bolus of ACh was injected approximately 1cm above the coronary vessels. Responses to acetylcholine occurred within 1–2s of injection. For a period of several minutes, flow was allowed to return to the previous control level prior to the administration of another dose of ACh. Atropine (0.1mg) was administered at the end of each experiment, followed by a previously effective dose of ACh.

Results

Rat hearts became unresponsive to stimulation and ceased to function between 17 and 12˚C (Fig. 1). By contrast, squirrel hearts continued to function at temperatures as low as 5˚C (Fig. 2). Between 37 and 15˚C, f_C decreased in hearts from both species and then remained relatively stable as temperature decreased from 15 to 5˚C (Fig. 3). At all temperatures, squirrel f_C was significantly higher than rat f_C (Fig. 3).

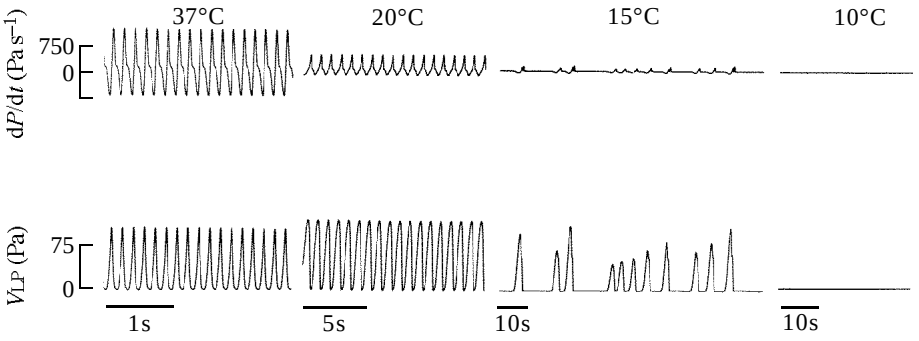

Fig. 1. The effect of temperature on contractility (dP/dt) and left ventricular systolic pressure (V_{LP}) in isolated rat hearts.

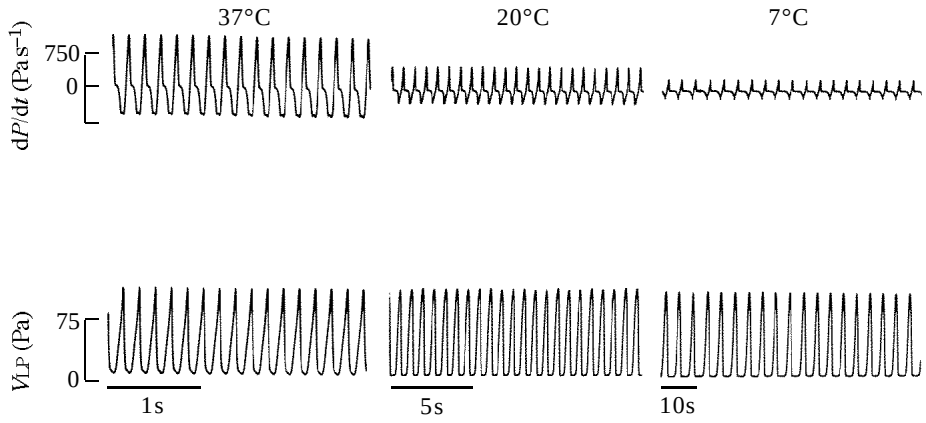

Fig. 2. The effect of temperature on contractility (dP/dt) and left ventricular systolic pressure (V_{LP}) in isolated ground squirrel hearts.

Acetylcholine increased f_C in squirrel hearts at 37˚C (Fig. 4). ACh also modified f_C at 20˚C but these changes were not significant. Squirrel heart f_C was unaffected by ACh at 7˚C. In rat hearts, f_C was reduced significantly by ACh at 37 and 20˚C (Fig. 5). After treatment with atropine, f_C remained unchanged when repeated doses of acetylcholine (>10 μg) were administered to rat or ground squirrel hearts. We interpreted this to be an insurmountable block of ACh receptors and no further experiments were performed. In every case, the antagonistic effect of atropine was independent of temperature.

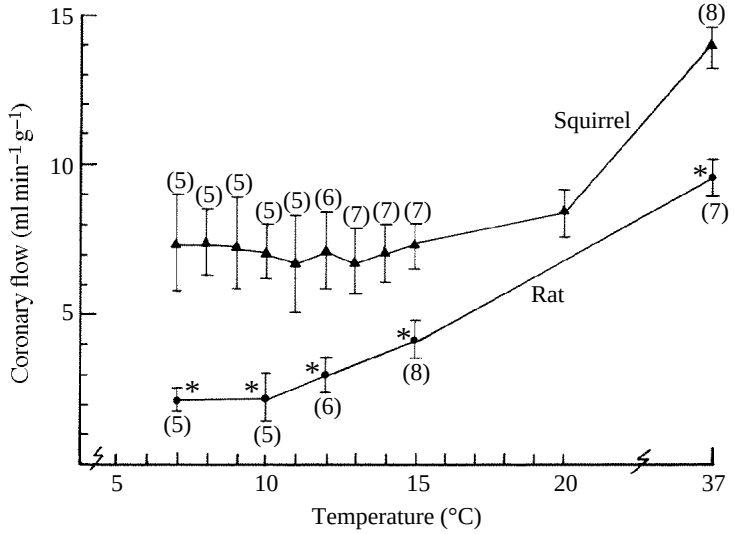

Fig. 3. The effect of temperature on coronary flow in rat (circles) and ground squirrel (triangles) hearts. Points represent mean ± S.E. for the number of observations in parentheses. Asterisks denote significant differences between means for the two species (P<0.01).

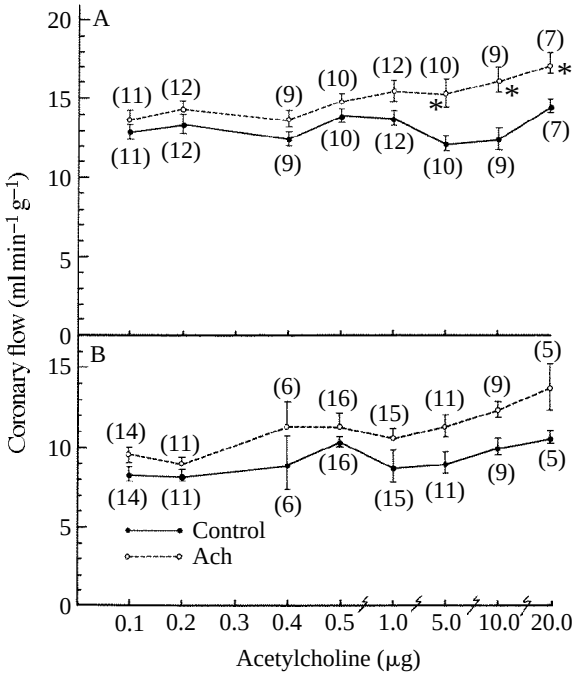

Fig. 4. The effect of different doses of acetylcholine (ACh) on coronary flow in isolated ground squirrel hearts at (A) 37˚C and (B) 20˚C. Points represent mean ± S.E. for the number of observations in parentheses. Asterisks denote significant differences from control values (P<0.01).

Similar results were obtained regardless of the condition of the ground squirrels (hibernating or nonhibernating) and whether the hearts were paced or beating spontaneously.

Discussion

The ability of squirrel hearts to function at temperatures ranging from 2 to 7˚C is well documented (Lyman, 1982; Burlington *et al.* 1986). Between 37 and 5˚C, O_2 consumption is closely matched to the product of heart rate and V_{LP} in squirrel hearts but not in rat hearts (Burlington *et al.* 1989). Furthermore, cardiac efficiency [energy output ($J\,g^{-1}$)]/[energy input ($J\,g^{-1}$)] increases in squirrel hearts at lower temperatures but it decreases in rat hearts over the same temperature range (Burlington and Darvish, 1988). The present data show a fall in f_C when the temperature drops from 37 to 20˚C, but a relatively constant f_C at temperatures between 20 and 7˚C in squirrel hearts. At these temperatures, squirrel f_C was significantly higher than that of rat hearts. Ground squirrel hearts have a greater capillary density and a decreased O_2 diffusion distance than do rat hearts (Burlington and Dean, 1983). Rat heart failure between 15 and 7˚C was observed during a previous study (Burlington and Zook, 1985). The above data support Thauer's (1965) contention that a deficit in f_C may be partially responsible for hypothermic cardiac failure in nonhibernating mammals.

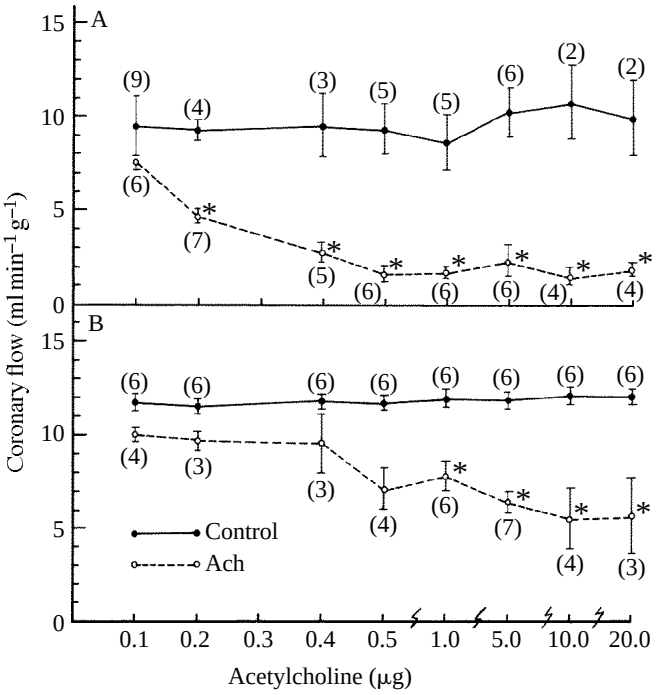

Fig. 5. The effect of different doses of acetylcholine (ACh) on coronary flow in isolated rat hearts at (A) 37˚C and (B) 20˚C. Points represent mean ± S.E. for the number of observations in parentheses. Asterisks denote significant differences from control values (P<0.01).

Until recently, the role of ACh in mediating f_C has been considered unimportant. The mammalian coronary vasculature is innervated by branches of both the sympathetic and parasympathetic nervous systems (Thomas *et al.* 1984). A growing body of evidence supports the hypothesis that neural control is important, but not essential, for the regulation of f_C (Kalsner, 1985). Vagal activation or treatment with ACh causes vasodilation of canine coronary arteries (Feigl, 1969, 1983; Cox *et al.* 1983; Reid *et al.* 1985). This may, in part, be mediated by an endothelium-derived relaxing factor (EDRF) liberated by the action of ACh on muscarinic receptors (Furchgott and Zawadski, 1980).

Studies of species other than dogs support an alternative viewpoint (Kalsner, 1989). ACh constricts human coronary arteries, even in the presence of an intact endothelium (Angus *et al.* 1991). Similar results have been obtained with coronary arteries from pigs (Cowan and McKenzie, 1990; Nakayama *et al.* 1988), sheep, cattle and monkeys (Kalsner, 1979, 1985). These effects are blocked by atropine and they indicate the presence of muscarinic constrictor receptors in the smooth muscle of coronary vessels. Therefore ‘constriction and not dilation may be the dominant vascular response to activation of the cholinergic axis in most mammals’ (Kalsner, 1989). Furthermore, ACh-induced coronary vasoconstriction has also been demonstrated in a diverse group of animals, including fish, amphibians, reptiles and birds (Kalsner, 1989). These observations suggest a phylogenetic continuity of the vasoconstriction response. Our data

from rat hearts support this hypothesis and confirm an earlier study by Sakai (1980). The combined results suggest that ACh primarily activates constrictor receptors in rat coronary artery smooth muscle. In this regard, the rat heart, unlike the canine heart, is a better model for the effect of ACh on human coronary vessels.

In contrast to the rat, ground squirrel coronary vessels are relatively unresponsive to ACh. Their response, if any, was dilation rather than constriction. This effect only became significant at doses of ACh exceeding 5 μg at 37˚C. ACh had virtually no effect on flow in squirrel hearts at 7˚C. In view of these data, it seems likely that muscarinic constrictor receptors are sparse in ground squirrel coronary vessels.

It also seems likely that cholinergic receptors play little or no role in regulating f_C during hibernation. Our finding that ACh had a diminished effect on f_C at 20˚C, and no effect at 7˚C, is supported by data from an *in vivo* study with ground squirrels (Milsom *et al.* 1988), which showed that the effects of vagal stimulation on heart rate and resting vagal tone decrease in parallel with decreasing temperature. There is, however, good autoregulation of f_C at 7–37˚C in isolated perfused ground squirrel hearts (Burlington *et al.* 1989). Therefore, the emerging picture is one wherein direct neural control of f_C is probably not a primary regulatory mechanism in the hibernating mammal. Rather, flow is probably controlled by local vasoactive factors from endothelial cells which are in turn regulated by pressure, flow, oxygen tension, etc. This hypothesis is consistent with current concepts regarding the role of endothelial mechanisms in control of vascular tone (Miller, 1991). In the rat, by contrast, direct neural control of f_C may be relatively important.

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