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Effects of prolonged cold storage on double peaked vasoconstrictor responses to periarterial nerve stimulation in isolated canine splenic arteries

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1 P2X-Purinoceptors and α_1-adrenoceptors have previously been shown to involve in the double peaked vasoconstrictor responses to periarterial electrical nerve stimulation in the isolated and perfused canine splenic artery. The present study made an attempt to investigate effects of prolonged cold storage (7 days at 4°C) on vasoconstrictor responses to periarterial electrical nerve stimulation, tyramine, noradrenaline and adenosine 5′-triphosphate (ATP) in the isolated canine splenic artery.

2 The periarterial nerve stimulation (1–10 Hz) readily causes a double peaked vasoconstriction in the non-stored preparations. After cold stored for 7 days, the double peaked vasoconstriction was still recognized, although the response became significantly smaller. The first phase was decreased relatively greater than the second phase by the cold storage.

3 In the cold stored preparations, the dose-response curve for tyramine was shifted to the right in a parallel manner. Prazosin almost completely inhibited tyramine-induced vasoconstriction but α,β-methylene ATP failed to influence the response to tyramine.

4 The vasoconstrictor responses to noradrenaline and ATP were not significantly modified by the prolonged cold storage.

5 From these results, it is concluded that the functions of sympathetic co-transmission of purinergic components might be influenced more than that of adrenergic components in the cold storage canine splenic artery.

Keywords: Cold storage; tyramine; sympathetic nerve stimulation; P2X-purinoceptor; splenic artery

Abbreviations: ATP, adenosine 5′-triphosphate; ES, periarterial electrical stimulation

Introduction

Biphasic vasoconstrictor responses to electrical nerve stimulation have been obtained in a variety of vessel preparations (Kennedy *et al*., 1986; Machaly *et al*., 1988; Bulloch & Starke, 1990; Sjöblom-Widfeldt *et al*., 1990; MacDonald *et al*., 1992). Recently, Yang & Chiba (1998) reported that purinergic and adrenergic components differently contributed to double peaked vasoconstrictor responses to periarterial electrical nerve stimulation in isolated and perfused canine splenic arteries. The first phase might contain mainly a purinergic component, and the second phase mainly an adrenergic one. It has been reported by several investigators (Shibata *et al*., 1970, 1971; Kristek *et al*., 1993) that prolonged cold storage causes irreversible degeneration of adrenergic nerve fibres and this abolishes adrenergic transmission. However, Ito & Chiba (1985) demonstrated that a prolonged cold storage (5–7 days at 4°C) of the isolated canine intermediate auricular artery might not cause complete denervation of adrenergic nerve fibres but rather resistant to the cold storage. Moreover, Sinanovic & Chiba (1987) also demonstrated that tyramine-induced vasoconstrictor responses were not significantly modified after 3–9 days cold storage (4°C) in skeletal muscle branches of the dog femoral artery. Since adenosine 5′-triphosphate (ATP) had been proposed as a co-transmitter with noradrenaline in sympathetic nerves, we tried to compare the double peaked vasoconstrictor responses to periarterial electric nerve stimulation in the cold stored canine splenic arteries to responses in fresh preparations, in order to clarify whether a prolonged cold storage affects the sympathetic co-transmission of purinergic and adrenergic component.

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Methods

Arterial preparations

Mongrel dogs of either sex, weighing 8–16 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.). After treatment with sodium heparin (200 units kg^{-1} i.v.), the dogs were killed by rapid exsanguination from the right femoral artery. The arterial main branches of the splenic artery were isolated, and side branches of the artery were tied with silk threads. Then, the artery (1–2 mm in an outer diameter) was cut into segments (15–20 mm in length), and each segment was cannulated and set up for perfusion as described previously (Tsuji & Chiba, 1984; Chiba & Tsukada 1985; Ren *et al*., 1994). During cold storage in the refrigerator, the isolated artery was kept in the Krebs-Henseleit solution without supplying exogenous oxygen at a constant temperature of 4°C for 7 days. After the cold storing period, segments were cut from arteries and prepared for the experiments in the same manner as a fresh artery. Briefly, a stainless steel cannula was inserted into the arterial segment from the distal to the proximal end. A proximal portion of the segment was fixed to the distal portion of a needle-type cannula with silk threads. The cannula was 3–4 cm long and

0.8–1.8 mm in an outer diameter and had small side holes 5 mm from the distal sealed end. The cannulated arterial segment was placed in a cup-shaped glass bath and was perfused by a roller pump (Tokyo Rikakikai) with Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2. The solution contained (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 25, $NaHCO_3$ 25 and glucose 10. The flow rate was kept at approximately 2 ml min^{-1}. The perfusion pressure was continuously measured with an electric manometer (Nihon Kohden, MPU-0.5A) and recorded with a rectigraph (Nihon Kohden, WT-685G). After a stabilization period of 60 min, the preparation was removed from the bath solution and fixed in a horizontal position. The preparation was perfused at a constant flow rate during the experiment. The basal perfusion pressure was within 35–80 mmHg.

For electrical stimulation of the periarterial sympathetic nerve terminals, two platinum electrodes were placed on the extraluminal side of the arterial wall. Electrical stimulation was delivered by an electric stimulator (SEN-7203, Nihon Kohden) using 30 s trains of pulses at 10 V amplitude, 1 ms pulse duration, in a frequency range of 1–10 Hz. The organ bath was sealed with the plastic film to maintain the preparation at 37°C. Ten min intervals between electrical stimulation periods were needed to obtain reproducible response. The intervals between frequency-response curves were 60 min. The drug solution was administered into the rubber tubing close to the cannula in a volume of 0.01–0.03 ml, by use of microinjectors.

Drugs

Drugs used were tyramine hydrochloride (Wako, Osaka, Japan); disodium adenosine 5′-triphosphate (ATP, Sigma, U.S.A.); noradrenaline hydrochloride (Sankyo, Tokyo, Japan); α,β-methylene ATP (Research Biochemicals International, Natick MA, U.S.A.); prazosin hydrochloride (Sigma). All drugs were dissolved in physiological saline before the start of the experiment. The stock solutions were kept at −20°C until used.

Statistical analysis

Vasoconstrictor responses to electrical stimulation or an agonist are expressed as the maximal changes in perfusion pressure (mmHg) from their control levels. The data are presented as mean ± s.e.mean. An analysis or variance with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. P values less then 0.05 were considered statistically significant.

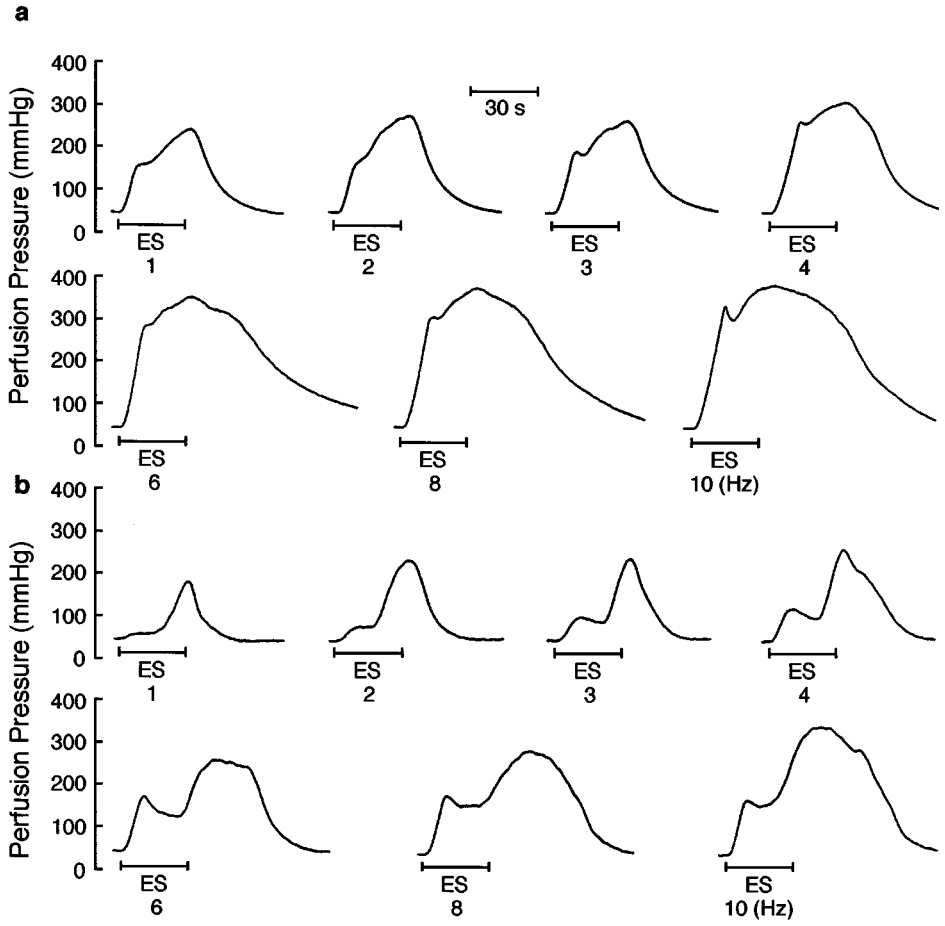

Figure 1 Double peaked vasoconstrictor responses of an isolated, perfused canine splenic artery preparation and the effect of prolonged cold storage (7 days at 4°C). The vessel was electrically stimulated by 30 s trains of pulses at 10 V amplitude and 1 ms pulses duration, with a frequency range of 1–10 Hz. (ES), Electrical stimulation. (a) Responses of a fresh artery, (b) responses of a cold stored artery (7 days at 4°C).

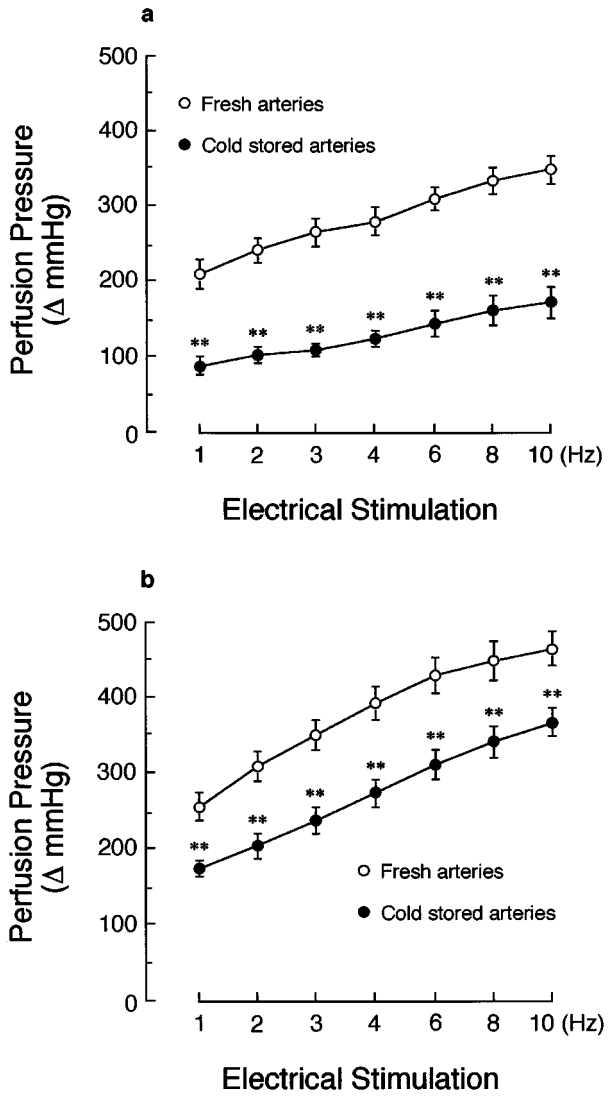

Figure 2 Effects of a prolonged cold storage (7 days at 4°C) on the first peak (a) and the second peak (b) of the biphasic vasoconstrictor responses to electrical stimulation (10 V amplitude, 1 ms pulse duration and 30 s trains of pulses at stated frequencies) in the canine splenic artery. Data are presented as mean ± s.e.mean ($n=8$). $^{**}P<0.01$ as compared with the fresh group.

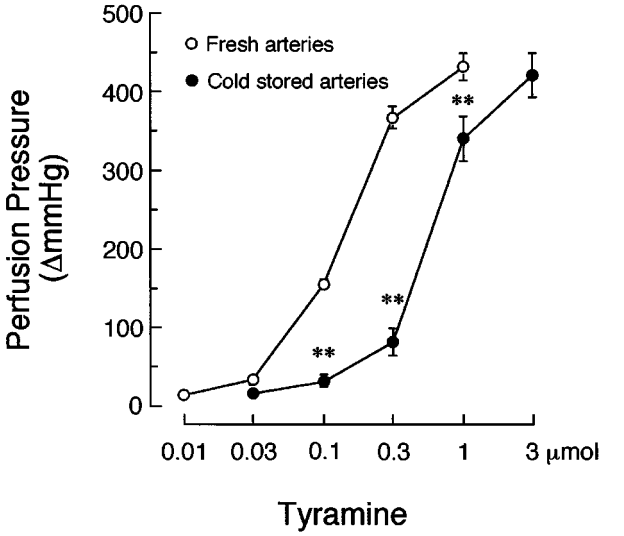

Figure 3 Effects of a prolonged cold storage (7 days at 4°C) on the vasoconstrictor response to tyramine in the isolated canine splenic artery. Data are presented as mean ± s.e.mean ($n=8$). $^{**}P<0.01$ as compared with the fresh group.

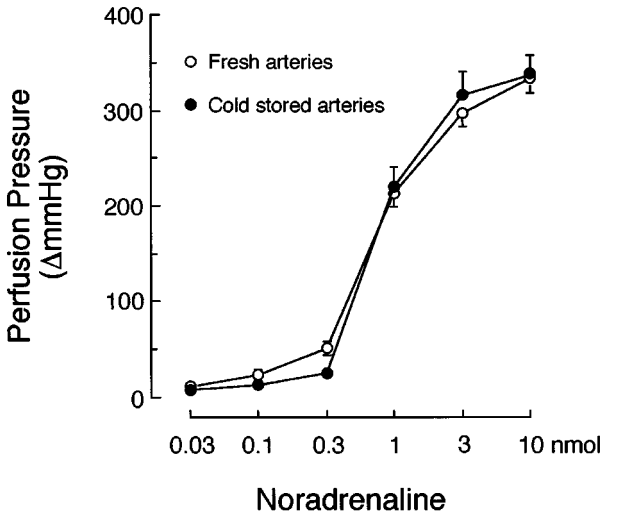

Figure 4 Effects of a prolonged cold storage (7 days at 4°C) on the vasoconstrictor response to noradrenaline in the isolated canine splenic artery. Data are presented as mean ± s.e.mean ($n=6$).

Results

Vascular responses to periarterial electrical nerve stimulation

The periarterial electrical stimulation (30 s trains of pulses) induced a double peaked vasoconstriction (2 phases of the vasoconstriction) in the isolated and perfused canine splenic artery in a frequency-related manner and usually separated by an intervening dip in increasing perfusion pressure (Figure 1a) as reported previously (Yang & Chiba, 1998). The cold storage for 7 days (4°C) caused to depress the first phase response to periarterial electrical nerve stimulation. On the other hand, the second phase was rather comparatively resistant to the cold storage, although both phases were significantly depressed (Figure 1b). Figure 2 shows summarized data of effects of prolonged cold storage on the double peaked vasoconstrictions (a: first phase, b: second phase).

Vascular responses to tyramine

When tyramine (0.01 – 3 μmol) was intraluminally administered in these preparations, vasoconstrictions were produced in a dose-related manner. The tyramine-induced response was almost completely inhibited by an α_1-adrenoceptor antagonist, prazosin (0.1 μM), but not influenced by a P2X-purinoceptor desensitizer, α,β-methylene ATP (1 μM) (data not shown). Figure 3 shows the vasoconstrictor responses of fresh and cold stored isolated canine splenic arteries to tyramine. In the fresh preparations, the contractile threshold concentration of tyramine was approximately 0.01 and 0.3 μmol of tyramine caused a strong vasoconstriction such as over 300 mmHg. On the other hand, in cold stored preparations, the threshold concentration was approximately 0.03, and 1 μmol of tyramine also induced a marked contractile response over 300 mmHg.

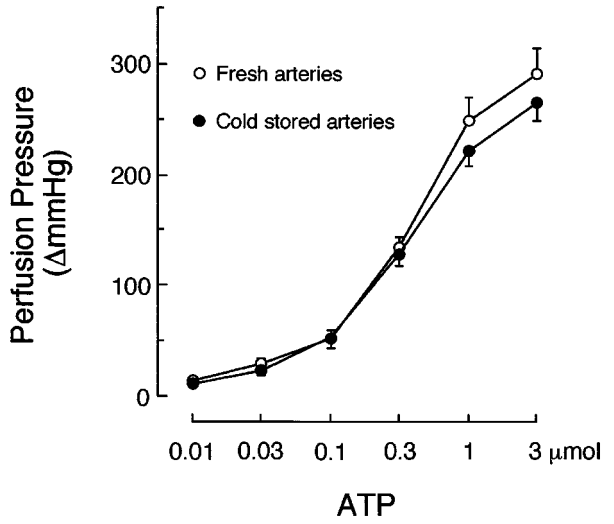

Figure 5 Effects of a prolonged cold storage (7 days at 4°C) on the vasoconstrictor response to ATP in the isolated canine splenic artery. Data are presented as mean ± s.e.mean ($n = 6$).

This shows that the concentration-response curve for tyramine is shifted to the right in almost a parallel manner, indicating that the responses to tyramine are three times less potent in the cold storage.

Vascular responses in noradrenaline and ATP

When noradrenaline (0.03 – 10 nmol) was intraluminally administered, marked vasoconstrictions were consistently induced in a dose-related manner. The dose-response curve for noradrenaline is almost the same in both fresh and cold storage preparations as shown in Figure 4.

When a relatively large dose of ATP (0.01 – 3 μmol) was given intraluminally, marked vasoconstrictions were also introduced in a dose-related manner. The dose-response curve for ATP is almost the same in both fresh and cold storage preparations as shown in Figure 5.

Discussion

It had been believed for a long time that a prolonged cold storage causes an irreversible degeneration of adrenergic nerve fibres leading to a depletion of tissue catecholamines (Shibata *et al*., 1970, 1971, 1977). In 1993, Kristek *et al*. reported that contractions of aortic rings in rabbits in response to transmural nerve stimulation was entirely abolished after 6 days cold storage (4°C). However, the present study showed that cold storing for 7 days did not abolish a double peaked vasoconstriction in an isolated canine splenic artery, remaining over 50% constrictor responses to periarterial nerve stimulation. The tyramine-induced vasoconstriction was shifted to the right in a parallel manner after 7 days cold storage. However, a marked contractile responses to higher doses of tyramine were still produced, showing the maximum constrictor response such as 300 mmHg. These results indicated that sympathetic nerve terminals remain to function even in cold stored preparations, as reported by Ito & Chiba (1985) using the isolated and perfused canine intermediate auricular artery.

We recently demonstrated that P2X-purinoceptor and α_1-adrenoceptor mechanisms involve the double peaked vasoconstrictor responses to periarterial electrical nerve stimulation in the isolated canine splenic artery, i.e. the first phase response might be induced mainly *via* an activation of P2X-purinoceptors, and the second phase response might involve mostly in α_1-adrenoceptors and partially in P2X-purinoceptors (Yang & Chiba 1998). In the present study, after cold storing for 7 days, the first phase response was strongly depressed, whereas the second one was rather slightly depressed although it is significant. Since the vasoconstrictor responses to noradrenaline and ATP were not influenced by the prolonged cold storage, postsynaptic processes of adrenergic and purinergic components might not be modified. Therefore, we considered that the cold storage may dominantly injure the purinergic components presynaptically, and it appears that an inhibition of the purinergic component is much greater than that of the adrenergic components.

Driessen *et al*., (1996) reported that tyramine did not release neuronal ATP as a co-transmitter of noradrenaline in the guinea-pig vas deferens. In this study, we observed that vasoconstriction induced by tyramine was almost entirely inhibited by prazosin, but not modified by α,β-methylene ATP, indicating the responsiveness to tyramine would only represent a function of sympathetic co-transmission of adrenergic component. The second phase response to electrical nerve stimulation might be mainly due to released noradrenaline as shown previously (Yang & Chiba, 1998), and that is rather resistant to the cold storage more than the first phase response. Therefore, it is reasonable to recognize that a co-transmission of the adrenergic component in the isolated cold stored canine splenic artery was still remained, because the response to tyramine was well produced even after 7 days cold storage.

The effects of a cold storage on the vasoconstrictor responses to exogenous noradrenaline have been observed in the rabbit aorta (Shibata *et al*., 1969), canine femoral artery (Murphy *et al*., 1973), and they indicated the potentiating effect of cold storage on the noradrenaline-induced constriction. Opposite results have also been obtained in the rabbit aorta (Varma & McCullough, 1969), and canine and monkey skeletal muscle arteries (Sinanovic & Chiba, 1987, 1988). In the present study, we failed to observe significant influence of cold storage on the noradrenaline and ATP-induced vasoconstriction, suggesting that postsynaptic responsiveness to noradrenaline and ATP might well be remained after 7 days cold stored in the isolated canine splenic artery. This discrepancy may probably be due to species differences, different procedures, different sizes of blood vessel preparations, different organ vessel and so on.

It is concluded that the prolonged cold storage of vessels may preferentially depress the co-transmission of purinergic component, whereas adrenergic component might still largely remain in isolated canine splenic artery.

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