Responsiveness of Monkey Skeletal Muscle Arteries to Vasoconstrictor Substances before and after Cold Storage

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Abstract—The stainless steel cannula inserting method was used to observe effects of α-adrenoceptor agonists, 5-HT and KCl before and after cold storage (3–5 days, at 4°C) in skeletal muscle branches of the simian deep femoral artery. Epinephrine (EPI), norepinephrine (NE), phenylephrine (PE), methoxamine (MT) and 5-hydroxytryptamine (5-HT) induced marked monophasic vasoconstrictions in a dose-dependent manner. 5-HT induced a greater vasoconstriction in larger diameter vessels (old animals) than that in smaller ones (young animals), suggesting age-related responses. A selective α2-stimulant, clonidine (CLO) or xylazine (XYL), produced only a slight vasoconstriction. Tyramine (TYR) also produced only a slight vasoconstrictor response. The order of potencies for inducing vasoconstrictions was EPI > 5-HT > NE > MT = PE > KCl = CLO = XYL = TYR. The vasoconstrictor responses to all used adrenergic agonists and 5-HT were not significantly influenced by the prolonged cold storage. However, KCl-induced constrictions were significantly suppressed by the cold storage. These results suggest that the postjunctional α-adrenoceptor in simian skeletal muscle arteries is mainly of the α1-type. Since cold storage caused a significant suppression of the KCl-induced response but not those of adrenoceptor agonists and 5-HT, it was considered that the mechanism of calcium entry to the vascular smooth muscle of skeletal muscle arteries might be significantly damaged by the cold storage.

It is well-known that the vascular responsiveness to various vasoactive substances is influenced by many factors, i.e., species, localizations, size, age and different experimental methods. The circulation in skeletal muscle has been investigated mostly in in vivo studies (1–5). There is, however, only scant literature available on the reactivity in response to vasoactive substances in isolated skeletal muscle arteries (6, 7).

It has been reported that the cannula inserting method is useful for investigating responses of both relatively small and large isolated vessels to vasoactive substances (8, 9). By the use of this method, we previously reported vascular responses to α-adrenoceptor stimulants of branches of the canine femoral artery which supply blood mainly to the skeletal muscle (10). We demonstrated that the postjunctional α-adrenoceptor in these dog vessels is of the α1-type and that cold storage caused a much greater suppression of KCl-induced vasoconstrictions than α-adrenoceptor agonist-induced ones.

Although there are many studies on the effects of various vasoactive substances on regionally different vessels of monkeys, there is still no available report on the vascular reactions of skeletal muscle arteries in the monkey. Thus, the purpose of this study was to investigate by the cannula inserting method the vascular reactivity of the branches of the simian deep femoral artery which are distributed in the skeletal muscle. We also ex-

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examined the effects of cold storage (3–5 days, at 4°C) on the vascular reactivity of these arteries.

**Materials and Methods**

Twenty-one Japanese monkeys (*Macaca Fuscata*) weighing 2.8–23 kg were anesthetized with ketamine hydrochloride (10 mg/kg, i.m.) and killed by exsanguination after treatment with sodium heparin (200 units/kg, i.v.). Branches of the deep femoral artery, which supply blood flow to skeletal muscle (Fig. 1), were carefully isolated, and the branch was cut into several segments (0.5–0.8 mm in outer diameter and 5–10 mm in length). Then, each segment was cannulated and set up for perfusion as described previously (8, 9). During cold storage in the refrigerator, the isolated artery was kept in the Krebs’ solution without supplying exogenous oxygen, at a constant temperature of 4°C for 3–5 days. The pH of the Krebs’ solution was about 7.7 during the storage.

After the cold storing period, segments were cut from the arteries and prepared for the experiments in the same manner as a non-cold stored artery. Briefly, a stainless steel cannula (25 or 27 gauge, 0.4–0.9 mm in outer diameter and 2.5–3 cm in length) with one or two small holes at a distance of 5 mm from the distal blind end, was carefully inserted into each vessel segment. The segment was tied to the cannula distal from the hole, and thus the stream from the hole of the cannula passed only through the intraluminal surface of the vascular segment. As previously reported in this laboratory, the endothelium was demonstrated to be intact by histological study after inserting the cannula into the vessel segments (11, 12). The isolated, cannulated artery was placed in the bath, which was maintained at a temperature of 37°C by a circulator thermopump (Haake FE2). The arteries were perfused with Krebs’ solution (millimolar: NaCl, 118; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgCl₂, 1.2; NaHCO₃, 25; and glucose, 5.6) and gassed with 95% O₂ and 5% CO₂ maintaining the pH levels at 7.2–7.4. The flow rate was initially adjusted (1–2 ml/min) so that the perfusion pressure was between 50–100 mmHg, and then it was kept constant throughout the experiment. The perfusion pressure was measured with an electro-manometer (Nihon Kohden, MPU-0.5A), and a vasoconstriction was recorded as an increase in perfusion pressure. Before the start of the experiments, all preparations were allowed to equilibrate for over 1 hr in the bathing medium. The volume of drug solution in a single injection was 0.01–0.03 ml, which was administered by a microsyringe (Terumo Co.). Since the agonist was administered by a bolus injection, the concentration was not equilibrated during the experiments. However, as the perfusion flow rate was constant and absolute doses of the agonist were confirmed, it was roughly calculated and can be compared with the results in different in vitro experiments. The interval between drug administrations was greater than 4 min in order to prevent tachyphylaxis.

Drugs used were dl-epinephrine hydrochloride (EPI, Sankyo), dl-norepinephrine hydrochloride (NE, Sankyo), phenylephrine hydrochloride (PE, Kowa), methoxamine hydro-

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**Fig. 1.** Schematic illustration of most frequent vascular distribution of the simian femoral arteries. 1. a. profunda femoris; 2. a. femoralis; 3. a. circumflexa ilium superficialis; 4. a. circumflexa femoris lateralis; 5. a. circumflexa femoris medialis; 6. m. iliopsoas; 7. m. sartorius; 8. m. adductor longus; 9. m. rectus femoris; 10. m. adductor magnus. Arrows show the skeletal muscle branches used in this study.
chloride (MT, Bayer), clonidine hydrochloride (CLO, Boehringer Ingelheim), xylazine hydrochloride (XYL, Bayer), tyramine hydrochloride (TYR, Tokyo Kasei), serotonin creatinine sulfate (5-hydroxytryptamine, 5-HT, Sandoz) and potassium chloride (KCI, Wako).

The data are expressed as means±S.E.M. in the text and illustrations, and significance of the differences between mean values was obtained by Student’s t-test for unpaired variates.

Results

Vascular responses to adrenergic agonists in fresh arterial preparations: Tracings of typical responses for the used adrenergic agents are shown in Fig. 2, and summarized data of the dose-response curves for all used vasoactive substances are shown in Fig. 3. Mixed α-adrenoceptor agonists, EPI and NE, and α1-adrenoceptor agonists, PE and MT, produced an immediate and transient increase in perfusion pressure in a dose-dependent manner. Threshold doses for inducing vasoconstriction were 0.001–0.003 μg for EPI and NE and 0.1–0.3 μg for PE and MT. A dose of 0.1 μg of EPI and NE usually produced a marked increase on perfusion pressure (more than 80 mmHg). On the other hand, α2-adrenoceptor agonists, CLO and XYL, and an indirect sympathomimetic amine, TYR, produced only a slight increase in perfusion pressure even in very large doses. Doses of
Fig. 3. The dose-response curves for 7 different \( \alpha \)-stimulants, 5-HT and KCl in isolated and perfused simian skeletal muscle branches of the deep femoral artery. Responses are expressed as values of absolute increases from the control level. Open and closed circles represent the mean values, and vertical bars represent S.E. Numbers of experiments are presented in parentheses. EPI, epinephrine; NE, norepinephrine; 5-HT, 5-hydroxytryptamine; PE, phenylephrine; MT, methoxamine; CLO, clonidine; XYL, xylazine; TYR, tyramine; KCl, potassium chloride.

Fig. 4. The dose-response curves for NE (A) and 5-HT (B) in large arteries (0.7-0.8 mm OD) from old monkeys and small arteries (0.5-0.6 mm OD) from young monkeys. Circles represent the mean values, and vertical bars represent S.E. *\( P<0.005 \), **\( P<0.001 \).
10 µg or 30 µg of CLO usually produced the maximum response. At doses over 30 µg, CLO-induced vasoconstriction became smaller, showing a bell-shaped dose-response curve. XYL frequently caused tachyphylaxis especially in large doses. The potency order for inducing vasoconstriction was EPI > NE > MT > PE > CLO = XYL = TYR. Vasoconstrictions by all used adrenergic agonists were not different between arteries from young animals (0.5–0.6 mm OD) and arteries from old animals (0.7–0.8 mm OD). This is exemplified by the NE-induced response in small and large arteries shown in Fig. 4A.

Vascular responses to 5-HT and KCl in fresh arterial preparations: 5-HT, intraluminally administered into the isolated artery, caused a strong monophasic vasoconstriction in a dose-related manner (Fig. 5A). The threshold dose for inducing a vasoconstrictor response in both arteries from young (0.5–0.6 mm OD) and old animals (0.7–0.8 mm OD) were different between arteries from young animals (0.5–0.6 mm OD) and arteries from old animals (0.7–0.8 mm OD). This is exemplified by the NE-induced response in small and large arteries shown in Fig. 4A.

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OD) was approximately 0.003 μg. However, vasoconstrictions induced by 5-HT in arteries from old animals were significantly greater than those in the vessels from young animals (P<0.001) for all used doses (Fig. 4B). A dose of 1 μg or 3 μg usually produced the maximum response. At larger doses over 3 μg, 5-HT-induced responses in both arteries from young and old animals became rather smaller, showing bell-shaped dose-response curves.

KCI induced a small vasoconstriction at larger doses (3 and 5 mg), and the maximum response to 5 mg of KCI was usually about 50 mmHg (Fig. 5A). The constrictor responses to KCI were not different between arteries from old and young animals. In 11 of 37 preparations, KCI did not produce any response by all used doses. The order of potencies for inducing vasoconstrictions for all drugs used in this study was EPI > 5-HT > NE > MT = PE > KCI > CLO = XYL = TYR.

Comparison of vascular responses to adrenoceptor agonists, 5-HT and KCI between fresh and 3–5 days cold-stored arteries: The responses to EPI, NE and MT were not significantly influenced by cold storage for 3–5 days. The dose-response curves for these drugs in both cold-stored and non-stored arteries are shown in Fig. 6.

The vasoconstrictor response to XYL and CLO also were not significantly different between non-cold stored and cold-stored preparations as shown in Fig. 7. However, in cold stored arteries, both CLO- and XYL-induced constrictions were occasionally followed by weak long-lasting dilation as shown in ○ ○ of Fig. 7.

The cold storage also did not change significantly vasoconstrictor responses to TYR and 5-HT as shown in Fig. 8. However, KCI-induced constriction was significantly decreased after 3 and 5 days cold storage, respectively (Fig. 9).

Discussion

The femoral artery of the Japanese monkey has no or a few very small and thin branches at the thigh level which is rich with muscle tissue. For this reason, we selected branches of a deep femoral artery which supply blood flow mainly to the thigh skeletal muscle, and they may represent skeletal muscle arteries. By use of these vessels, we demonstrated that the order of vasoconstrictor effects of α-adrenoceptor agonists was EPI ≥ NE ≥ MT = PE ≥ KCI ≥ CLO = XYL = TYR, which indicates that the postjunctional α-adrenoceptor in these arteries is mainly of the α1-type, although a definite conclusion should be made only after obtaining results by use of various α-adrenoceptor antagonists such as prazosin and yohimbine. These observations are almost the same as those shown previously to...
occur in dog skeletal muscle arteries (10), dog mesenteric arteries (13) and dog and monkey femoral arteries (14) by use of the same experimental procedure. In 1984, Chiba and Tsukada (15) reported that in the Japanese monkey mesenteric artery, the vasoconstrictor response to NE was almost the same as that in the dog. However, in the isolated simian femoral artery, the vasoconstrictor responses to α-adrenoceptor agonists were usually greater than those in the corresponding canine artery (14). We also observed greater responses to the same drugs used in present experiments than those in our previous study (10).

It is well-known that skeletal muscle vessels exhibit strong intrinsic basal tone and correspondingly weak nervous control (16). In 1985, Chiba and Ito (17) reported that TYR exerted its strong vasoconstrictor action in the dog intermediate auricular artery, but only a weak response in the dog mesenteric artery. In this study, the observation that rather large doses of TYR were necessary to induce a vasoconstriction indicates a poor sympathetic innervation or TYR-insensitive catecholamine storage in these preparations.

The majority of isolated blood vessels contracts when exposed to 5-HT, but large differences in sensitivity exist among vascular tissues from the different species, and within the same species, among blood vessels of different anatomical origin and different size (18–20). Haddy et al. (1) and Haddy (2) perfused the brachial artery of dogs, and they reported that 5-HT had a potent vasoconstrictor effect on the skin vascular beds, but
had no such effect on the vascular beds of skeletal muscle. In the present study, the threshold dose of 5-HT was also the same as that of NE, but the maximal response to 5-HT was slightly less than that to NE. Based on the arterial diameter, preparations of monkey skeletal muscle arteries were divided into two groups, i.e., arteries from young and old animals. In the arteries from young animals (0.5-0.6 mm OD), 5-HT showed much less efficacy than EPI and NE. On the other hand, in the arteries from old animals (0.7-0.8 mm OD), 5-HT caused a markedly greater response than EPI and NE. Arteries from old animals were isolated from monkeys weighing more than 10 kg. Exact ages of animals used in this study were not clear, but it is very likely that monkeys with the greater weight were older than small animals. The effects of age on the response of arteries to vasoactive substances have been well established (21-26). Recently, Tsujimoto et al. (26) reported that the vessels from the older rats were more sensitive to 5-HT, and the maximal 5-HT-induced contraction was greater in the ring segments from the older rats. In this study, the NE-induced constrictor response was not different between the two groups of vessels, i.e., old and young monkeys. It is likely the great vasoconstrictor response to 5-HT in the large artery is age-related, and due to increase in sensitivity to 5-HT of skeletal muscle arteries of aged animals.

In our previous experiments in skeletal muscle arteries of dogs, KCl produced relatively greater vasoconstrictor responses. It is well-known that KCl causes depolarization of the cell membrane which evokes contraction by enhancing the influx of calcium ions from the extracellular space through voltage sensitive calcium channels (27, 28). Thus, a relatively small vasoconstrictor response to KCl in skeletal muscle arteries may indicate the relative resistance of the cell membrane to the KCl depolarizing effect and consequently less Ca influx from the extracellular space, or/and an existence of relatively voltage insensitive Ca channels.

The effects of cold storage on the responsiveness of various vessels to α-adrenoceptor stimulants and other vasoactive substances were investigated by many authors, but the results have been rather controversial. Shibata (29) reported the potentiating effect of cold storage on NE- and EPI-induced vasoconstrictions in spiral strips of the rabbit aorta. Murphy et al. (7) also reported a similar result using perfused preparations of lateral branches of the dog femoral artery (which may supply blood to both the skeletal muscles and skin of the hindlimb). On the other hand, Varma and McCullough (30) showed a decreased sensitivity of cold stored rabbit aortic strips to NE. Recently, Ito and Chiba (31), using the cannula inserting method, showed that the responsiveness of dog auricular arteries (which mainly supply blood to the skin) to several vasoactive substances (NE, TYR, 5-HT, PGF2α and KCl) remained almost the same as in non-stored vessels. In our recent study (10), using dog skeletal muscle arteries, we showed that cold storage caused much greater suppression of the KCl-induced response than the α-adrenoceptor agonist-induced one. In this study, cold storage did not modify vasoconstrictor response to α-adrenoceptor agonists and 5-HT, but KCl-induced response was significantly depressed. Since cold storage in this study caused significant suppression of the KCl-induced response, but not the vasoconstrictor response to α-adrenoceptor stimulants and 5-HT, it is suggested that the mechanism of Ca entry into skeletal muscle arteries may be significantly damaged by cold storage.

In cold stored preparations, we could observe that CLO- and XYL-induced vasoconstrictions at larger doses were occasionally followed by weak long-lasting vasodilations. Since the response was induced variably among individual preparations only at large doses, it was difficult to analyze the response pharmacologically.

The skeletal muscle circulation is directly controlled by intramuscular capillary vessels. In that sense, the branches of the deep femoral artery used in this study may not play a critical role for the regulation of skeletal muscle blood flow. However, since the used branches supply blood flow mainly to the skeletal muscle, we believe that pharmacological analyses of the responses of these arteries would provide some insight into the
mechanism involved in the regulation of the skeletal muscle circulation.

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