Effect of Prolonged Cold Storage on the Contractile Response of Strips of Rabbit Aorta to Various Agents

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ABSTRACT

After 2 days in cold storage (2.0 ± 0.5°C), the contractile response of spiral strips of rabbit aorta to low concentrations (10^{-6} to 10^{-10} M) of epinephrine and of norepinephrine was potentiated; after storage for 4 to 7 days, an increased contractile response to high concentrations (10^{-8} to 10^{-6} M) of these catecholamines was also observed. Reserpine-pretreated strips showed potentiation of epinephrine- and norepinephrine-induced contractions, but only after 2 to 7 days in cold storage. Another result of several days of cold storage was a decrease or loss of contractile response to nicotine (10^{-5} to 10^{-8} M), tyramine (10^{-4} to 10^{-6} M), and potassium (10 to 40 mM). Potentiation of the contractile response to epinephrine and to norepinephrine was also observed in potassium-depolarized strips at low temperature (19°C). After incubation of the depolarized or cold-stored strips in the Ca-free medium, no potentiation of epinephrine- or norepinephrine-induced contractions was observed. These results indicate that cold storage, up to 7 days, does not interfere with the activation system related to epinephrine- or norepinephrine-induced contractions. However, such treatment does affect the membrane system involved in potassium-induced contraction. The decreased response to nicotine and tyramine after several days of cold storage may be due to the unavailability of tissue catecholamine. No significant histopathologic changes were observed in any layer of the aorta after cold storage up to 7 days.

ADDITIONAL KEY WORDS epinephrine norepinephrine KCL reserpine-treated strip nicotine tyramine depolarized strip low temperature histopathologic change

Even though the actions of catecholamine and some other agents on vascular smooth muscle have been studied, our knowledge of the mechanism of action of these compounds is far from complete. It is known that after smooth muscle has been exposed to cold for hours or days, the responsiveness which was lost during cold storage is regained when it is rewarmed to body temperature (1-4). However, while investigating the relationship between temperature and mechanical activity of vascular smooth muscle of rabbit aortic strip, it was noticed that the contractile response of the strip to epinephrine and to norepinephrine was potentiated after prolonged cold storage (2°C) although the responses to excessive amounts of other drugs such as potassium, nicotine, and tyramine were decreased or abolished. As yet, no report appears to have been made on the influence of prolonged cold storage (more than several days) on the contractile response of vascular smooth muscle to chemical stimuli. Because this phenomenon might help to elucidate the mechanism and site of actions of such stim-
ul as well as the mechanism of the effect itself, the modification of the contractile response to norepinephrine, epinephrine, potassium, nicotine, and tyramine by prolonged cold storage was studied.

**Methods**

For these studies, 185 rabbits of both sexes weighing 1.5 to 2.0 g were killed by a blow on the head. The carotids were cut and the chest opened. The thoracic aorta was removed and placed in Ringer's solution. Spiral aortic strips were prepared after excessive fat and connective tissue had been removed (5). A strip of aorta from each rabbit was cut transversely and one half used as a control for the other half. Strips 4 to 5 mm wide and 60 mm long were used in the experiments. The aortas were mounted vertically in an organ bath in 15 ml of Ringer's solution of the following composition (mM): NaCl, 154; KCl, 5.4; CaCl2, 2.4; NaHCO3, 6; dextrose, 11, all in distilled and deionized water. The solutions were maintained at 37°C and equilibrated before and during the experiment with a gas mixture of 95% O2 and 5% CO2 (pH 7.3) in the tissue bath. Ligatures were placed in both ends of the muscle strips, one being attached to a glass rod (holding device) and the other to a Grass FT .03 strain gauge.

During cold storage in the refrigerator, the strips were kept in the medium without supplying exogenous oxygen, at 2.0 ± 0.5°C for 1 to 12 days. Then strips were transferred to warmed Ringer's solution (37°C) and 1.5 g of tension was applied to the strips for 2 hours. The loaded tension was maintained throughout the experiment and it was periodically readjusted. Similar procedures were carried out on the control preparation, which did not have the cold-storage treatment.

During cold storage, there was no change in tissue medium nor was tension applied to the strip. Isometric contractions were recorded by a strain-gauge transducer (Grass FT .03) and 4-channel Grass polygraph. With this equipment, it was possible to examine four preparations in different tissue baths at the same time. The strips were exposed to various doses of epinephrine, norepinephrine, KCl, tyramine, and nicotine in normal or modified Ringer's solution.

Reserpine-pretreated strips were obtained from rabbits that had been injected with reserpine (Serpasil, Ciba), 4 mg/kg im, 24 hours before death. All rabbits treated with reserpine showed signs of effective treatment, such as diarrhea, ptosis and a reduction in heart rate.

Data is presented and analyzed in grams of contraction. Dose-response relationships were obtained by adding cumulative concentrations of the drug to the tissue bath except in the case of nicotine. The volume of each single addition was 0.5 ml. At least 1 hour was allowed between completion of one tissue stimulation and the beginning of the next to ensure uniform return to the previous resting tone.

All drugs were prepared immediately before use from concentrated stock solutions that were kept in a refrigerator. These solutions were made with distilled and deionized water. The concentrations of drugs tested are expressed as the final concentration in tissue bath. Ca-free medium refers to the normal Ringer's solution from which the calcium chloride was omitted.

**Results**

**RESPONSE TO EPINEPHRINE AND TO NOREPINEPHRINE**

Figure 1 shows the contractile response of the aortic strip to epinephrine and to norepinephrine after different durations of cold storage at 2°C. When the strips were rewarmed at 37°C in Ringer's solution, they regained the responsiveness to these agents they had lost at low temperatures. After 2 days in cold storage, only the contractile responses to low concentrations of epinephrine or norepinephrine (10^{-9} to 10^{-14}M) were potentiated (P < .01, t-test). In the fresh preparation (not cold stored) 10^{-10}M epinephrine or norepinephrine produced no contraction. The minimal effective dose of epinephrine and of norepinephrine producing a detectable contraction in control preparations was approximately 10^{-6}M. After 4 days in cold storage, potentiated responses were also seen with high concentrations of these catecholamines (10^{-6} to 10^{-8}M) (P < .02, t-test). This potentiation was observed even after 7 days in cold storage, but after 10 days, potentiation had disappeared and the response was decreased. High concentration of these drugs (10^{-6} to 10^{-8}M) could still cause a detectable contraction in any of seven strips from different rabbits after 20 days in cold storage, but the amplitude of the contractions was only 25% to 30% of the size of those in the fresh preparation. After 30-minute incubation in Ringer's solution containing desipramine hydrochloride (10^{-6} to 10^{-8}M) or monoamine oxidase inhibitors such
Changes in the contractile response of rabbit aortic strip to epinephrine and to norepinephrine after cold storage. The numbers in parentheses are the number of strips tested. The vertical lines represent the mean ± SD. The contractile activity on day zero is a control response determined before cold storage.
Effects of Various Agents on the Contractile Response to Epinephrine and to Norepinephrine

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Concentration (log M)</th>
<th>No. strips</th>
<th>EP (log M)</th>
<th>Contractile response (g) to stimulant</th>
<th>NE (log M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desipramine hydrochloride</td>
<td>6  5</td>
<td></td>
<td>2.8 ± 0.2</td>
<td>1.0 ± 0.2*</td>
<td>3.0 ± 0.16</td>
</tr>
<tr>
<td>Pheniprazine</td>
<td>5  5</td>
<td></td>
<td>2.8 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>3.0 ± 0.16</td>
</tr>
</tbody>
</table>

Before exposure to cumulative concentrations of epinephrine (EP) or norepinephrine (NE), the strips were incubated in Ringer's solution containing a pretreatment agent for 30 minutes. Contractile response expressed as mean ± SEM.

*P value is significant difference from control value (< 0.01 t-test).

The contractile response to epinephrine and to norepinephrine in response to cold storage and after cold storage from different rabbits were used in each experiment. The vertical lines represent SEM of the observations.

The time of potentiation after cold storage was the same in strips treated with reserpine as those not so treated. Figure 2 allows the use of transylcypromine hydrochloride (10⁻⁴ to 10⁻⁵M) or pheniprazine hydrochloride (10⁻⁵ to 10⁻⁶M) to potentiate the epinephrine and norepinephrine effects. These agents had no effect on the contractile response to epinephrine and norepinephrine effects. The time of potentiation after cold storage was only after 2 to 7 days in cold storage.
dose response curves of epinephrine and of norepinephrine (10⁻⁷ to 10⁻³ M) in strips from reserpine-treated rabbits before and after cold storage (4 days).

The histopathologic changes in aortic strips after cold storage were also studied. In each experiment, four aortic strips from different rabbits were used. The preparations were fixed with 10% neutral formalin, embedded in paraffin, and stained with hematoxylin-eosin. They were then cut in longitudinal and cross sections (4µ) using a rotary microtome, and histopathologic changes were examined under light microscope. No significant histopathologic changes were observed in any layer of the aortic strip after prolonged cold storage for up to 7 days. However, after 10 days in cold storage, marked vacuolization of the intra- and intercellular space in the media (muscle layer) was observed. These changes presumably explain the decreased contractile responses to epinephrine and to norepinephrine after 10 days in cold storage.

RESPONSE TO KCI

Figure 3 shows the contractile response to KCl (10 to 40 mM) after up to 7 days in cold storage. After 2 days in cold storage, the response to low concentrations of KCl (10 to 20 mM) was already decreased. After 4 days in cold storage, the response to high concentrations of KCl (30 to 40 mM) was also diminished. In fresh preparations, 40 mM KCl caused almost maximal contraction. No further contraction was observed after addition to 80 mM KCl solution. After 7 days in cold storage, a decrease in the contractile response to the high concentrations of potassium (40 mM) was observed.

After 7 days of cold storage in Ringer's solution containing excess potassium (15 mM), depression of the contractile response to potassium (40 mM) was significantly less than that after 7 to 10 days of cold storage in medium without excess potassium (Fig. 2). After 1-hour incubation in Ca-free medium, potassium-induced contraction (20 to 40 mM) was almost completely abolished, as shown in Figure 3.

When the four strips from different rabbits were incubated in Ringer's solution containing potassium (40 mM) for as long as 3 to 5 days in cold storage, the strips completely lost their responsiveness to potassium (80 mM). This suggests that such treatment may damage the vascular tissue, since even the application of BaCl₂ (5 mM) into the tissue bath could not bring about contraction in these preparations. In normal Ringer's solution, the amplitudes of the maximal contractions produced by 5 mM BaCl₂ were 4.1 ± 0.5 g and 3.9 ± 0.5 g in four fresh and four cold-stored (4 days) strips respectively.

RESPONSE TO NICOTINE AND TYRAMINE

Since nicotine and tyramine are believed to cause contraction of vascular smooth muscle by an indirect action involving liberation of norepinephrine from its tissue storage site, the action of these drugs on the preparation before and after different durations of cold storage was tested. Figure 4 shows the effect of cold storage on nicotine-and tyramine-induced contractions. After 24 hours of cold storage, the response to low concentrations of nicotine and tyramine (all 10⁻⁶ M) was...
decreased. After 3 to 6 days of cold storage, the responses to higher concentrations of nicotine ($10^{-5}$ and $5 \times 10^{-6}$M) and tyramine ($10^{-4}$ to $10^{-3}$M) were almost completely inhibited. After 10-minute incubation in a medium containing alpha-receptor blocking agents (phenoxylbenzamine hydrochloride and phentolamine methanesulfonate, both $5 \times 10^{-7}$M), the contractile response to nicotine ($10^{-5}$M) and tyramine ($10^{-4}$M) was almost completely diminished in all of five fresh preparations from different rabbits. Also, the contractile response to both drugs in strips from reserpine-treated rabbits was studied. The contractile responses of these to nicotine ($10^{-5}$M) and to tyramine ($10^{-4}$M) (10 preparations each) were diminished to about 30% to 40% of those in the untreated preparation.

**RESPONSE TO EPINEPHRINE AND NOREPINEPHRINE OF DEPOLARIZED PREPARATION**

Since at 37°C, excess potassium (40 mM) caused almost maximal contraction, the contractile response of the potassium-depolarized preparation to norepinephrine and to epinephrine could not be measured at that temperature. Therefore a reduced temperature (19°C) that prevented the contractile response to potassium but did not completely prevent the response to either epinephrine or norepinephrine was used in this experiment. The fresh preparation was depolarized by incubation in a high potassium medium (80 mM) at 19°C for 2 hours before the start of the experiment.

Figure 5 shows that the cumulative dose response curves for epinephrine and norepinephrine on the depolarized muscle were
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The contractile response of fresh strips and strips in cold for 6 days to epinephrine (EP) and norepinephrine (NE) in Ca-free medium with or without EDTA (0.1 mM). The strip was incubated in Ca-free medium for 1 hour. The vertical lines represent SEM of 10 observations.

Discussion

The mechanisms of the modification of responsiveness by prolonged cold storage is not clear; however, the present results indicate several possibilities. It was found, for example, that reserpine-pretreated strips did not show supersensitivity to epinephrine or to norepinephrine, except after prolonged cold storage. Also, the time for development of supersensitivity after cold storage was identical in both reserpine-treated and untreated strips. This might argue against supersensitivity as a depletion or releasing phenomenon of tissue catecholamine stores.

Neither treatment with desipramine, which prevents catecholamine uptake from the tissue storage site, nor treatment with monoaminooxidase inhibitors potentiated the response to epinephrine and norepinephrine in fresh aortic strips. Thus, it seems unlikely that supersensitivity is caused by increased availability of catecholamine to the effector cell as a consequence of the inhibition of uptake or breakdown of the catecholamines. I believe, however, that further study is necessary to rule out either of these possibilities completely.

It was also observed that supersensitivity to epinephrine and to norepinephrine occurred in potassium-depolarized fresh strips at low temperature. Aebi (6) and Shanes (7) have indicated that cold storage will depolarize a membrane. In view of the similarity between the supersensitivity of depolarized fresh and cold-stored muscle, it seems possible that the same mechanism is involved in both cases. Also, previous findings suggested that the magnitude of smooth muscle contraction is closely related to increased Ca flux (8, 9). Therefore, if increased Ca$^{2+}$ permeability is regarded as a common mechanism of supersensitivity in
both potassium-depolarized and cold-stored muscle, it should be expected that supersensitivity would be affected by external Ca\(^{2+}\) concentrations. The lack of supersensitivity to epinephrine and to norepinephrine in the Ca-free medium, therefore seems to support the previous suggestion by Carrier and Shibata (10) that membrane permeability changes are causally related to the development of supersensitivity to catecholamines in aortic strips.

It is well known that potassium-induced contraction is probably mediated by membrane potential changes (11-15). In addition, since in Ca-free medium the strip lost responsiveness to potassium may support this possibility, since external Ca\(^{2+}\) ion is probably an essential factor for the maintenance of the electrical activity of smooth muscle (16-20). It may be suggested that normal levels of membrane permeability are required to initiate a full contractile response to excess potassium. It has also been reported that cold storage inhibits the repolarization process as well as the return of potassium to the intracellular space, and this inhibition becomes irreversible below a critical level (6, 7). It was therefore speculated that the observed decrease in the contractile response to potassium might be due to incomplete recovery of the intracellular potassium level following prolonged cold storage. This interpretation is consistent with the observation that prolonged cold storage in Ringer's solution containing excess potassium (15 mM) decreased the inhibition of the potassium-induced contraction, since such treatment might partially prevent the decrease of intracellular potassium during prolonged cold storage.

The present observation that reserpine-pretreatment and alpha-receptor blocking agents (phentolamine and phenoxybenzamine) decreased the response to nicotine and tyramine supports the suggestion that these agents act indirectly on vascular smooth muscle by releasing available catecholamines from tissue storage sites (21-23). It has also been reported that prolonged cold storage allows irreversible degeneration of isolated nerve tissue, and this may lead to depletion of the tissue catecholamines (24). The ineffectiveness of nicotine and tyramine on cold-stored strips may thus be due to the absence of available endogenous catecholamine in the tissue stores. The inhibitory action of cold storage (3 to 6 days) on tyramine- and nicotine-induced contractions was greater than the effect of prior treatment with reserpine. Innes (25) has suggested that tyramine might be releasing residual amounts of tissue catecholamine after treatment with reserpine. It may be postulated that cold storage caused a greater depletion of tissue catecholamines than prior treatment with reserpine. Thus, although these investigations have shed some light on the mechanism of the indirect action of tyramine, the postulated direct action of tyramine remains unexplained (26-27). Further experiments are being carried out to investigate this interesting phenomenon.

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