THE EFFECT OF COCAINE ON THE $^3$H-NOREPINEPHRINE UPTAKE BY COLD STORED AORTA FROM RABBIT

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Abstract—The effects of cold storage ($2^\circ$C) on the uptake of $^3$H-norepinephrine ($^3$H-NE) by the rabbit aortae were studied. The cold storage progressively decreased the uptake of norepinephrine by strips exposed to low concentration ($10^{-7}$ M) of $^3$H-NE. By the 7th day of storage, the uptake became constant and was not affected by further treatment. Cocaine ($10^{-5}$ M) or desipramine ($10^{-6}$ M) failed to decrease this residual norepinephrine uptake in the strips stored for more than 5 days. Although cocaine potentiated the norepinephrine response of aortae from young (5 wk.) and old (over 10 months) rabbits equally, the cocaine sensitive norepinephrine uptake of old rabbit aortae was approx. half the value observed in the aortae of young rabbits. Study on $^3$H-metabolites indicated that the major portion of the radioactivity in the fresh and cold stored tissue was $^3$H-NE. The content of total $^3$H-metabolites formed by monoamine oxidase was less in the medium for the cold stored tissue than for the fresh tissue. These results indicate that cocaine sensitive neuronal uptake of norepinephrine by the rabbit aortae disappears after cold storage. These studies, together with previous experiments, strongly support the view that the cocaine induced potentiation in the aorta involves a postsynaptic component.

Inhibition of catecholamine uptake into nerve endings and the consequent elevation of catecholamine concentration at the receptor site is presumed to be the mechanism by which cocaine potentiates the response to norepinephrine (1). Several investigators, however, have recently suggested that the sensitizing action of cocaine on the norepinephrine response of vascular and other smooth muscles cannot be attributed entirely to inhibition of neuronal uptake of norepinephrine (2-8). Evidence to suggest this hypothesis is that cocaine still potentiates the vascular smooth muscle response to norepinephrine even after prolonged cold storage, which presumably causes functional disintegration or degeneration of adrenergic nerve terminals in the tissue (5, 7, 9). On the other hand, Graefe and Trendelenburg, (10) using cat nictitating membrane, concluded that cold storage of the muscle abolishes neither the neuronal uptake of norepinephrine nor the ability of cocaine to impair this uptake. Therefore, the basic question still remains as to whether prolonged cold storage leads to impairment of norepinephrine uptake by the nerve terminal in vascular smooth muscle. The present experiments were specifically designed to elucidate this question: to determine the ability of cold stored aortic strips to take up norepinephrine and further to study the effect of cocaine on the uptake mechanisms.

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MATERIALS AND METHODS
Young, male (about 1.2 kg, 5 wk. old) and when necessary, older male (3.5-4.0 kg, over 10 months old) rabbits were used in this study. They were sacrificed by a blow on the head, the carotids cut and the thoracic aortae removed. Spiral aortic strips were prepared after removal of excessive fat and connective tissue. The spiral strips from each rabbit were cut transversely into three pieces, one to serve as non-treatment control for the group. The strips were allowed to equilibrate in 15 ml Krebs-Ringer bicarbonate medium containing (in mM): Na+ 145, K+ 6.02, Ca++ 1.22, Mg++ 1.33, Cl 126, HCO3 25.3, PO4 1.2, SO4 1.33, and glucose 5.5, at 37°C for 2 hr before the experiments were started. For cold storage, the strips were stored in Krebs-Ringer bicarbonate medium at 2°C without a supply of oxygen for a period ranging from 1 to 15 days as described previously by Shibata et al. (7).

3H-Norepinephrine uptake and retention
To determine the radioactivity of tritiated norepinephrine (3H-NE) and tissue norepinephrine content, these procedures were followed: Tissues were incubated in l-3H-NE (6.6 c/mm mol or 2.18 c/mmol, Amersham/Searle Corp.) at 10^-7 M for 10 min or at 10^-5 M for 30 min (37°C) in 5 ml of Krebs solution. The incubation medium also contained ascorbic acid (0.2 mg/ml), bubbled with a mixture of 95% O2 and 5% CO2, adjusted to pH 7.3. The tissues were then immediately rinsed in fresh medium for 10 sec. In this experiment, the tritium radioactivity of strips after this procedure was taken as a measure of tissue uptake of 3H-NE. In other experiments, after exposure to 3H-NE (10^-7 M) for 10 min, tissues were incubated in fresh medium for 10 min. The tritium radioactivity retained by the tissues after this washing process designated the retention of 3H-NE. The radioactivity of the tissue after 10 min exposure to 3H-D-sorbitol (6.8 c/mm mol, New England Nuclear) was used to estimate the extracellular space of the tissue. To examine the correlation between the effect of cocaine on the norepinephrine uptake and on the sensitivity to norepinephrine under identical conditions, the experiment of uptake and sensitivity were performed on the strips from the same aorta. As reported in a previous study (7), cocaine (10^-6 M) developed supersensitivity to norepinephrine in cold stored strips.

The strips for radioisotopic determinations were blotted on filter paper, weighed and placed in 20 ml polyethylene vials. The tissues were digested with 0.5 ml of Soluene 100 (Packard) for 24 hr and dissolved in 15 ml mixture of scintillation fluid (2,5-diphenyloxazole; PPO, 5 g; 1,4-bis [2-(4-methyl-5-phenyloxazolyl)] benzene; dimethyl POPOP, 0.05 g; toluene, 1 liter). Aliquots of the incubation media were treated in a similar manner as the tissue. Total radioactivity was counted in a liquid scintillation spectrometer (Packard Tri-Carb). Each sample was counted for 10 min and the overall counting efficiency of our system was approx. 20.4%. Tissue radioactivity was expressed as ml of bath fluid cleared per gram wet wt. (ml/g). This expression is numerically the same as the tissue/medium ratio.

Analysis of 3H-NE and its metabolites
The tissue (200 mg) was incubated in 2 ml of Krebs medium containing 3H-NE (10^-7}
M) for 10 min, then rinsed 3 times with 5 ml of fresh medium and homogenized in acid-ethanol (0.1 ml concentrated HCl in 100 ml of ethanol) in order to extract radioactivity. The homogenate was centrifuged at 5000 × g for 10 min and an aliquot of the supernatant was applied to an acid-washed chromatographic filter paper. The medium was added to a chilled tube containing 0.2 ml of 0.5 N HCl and 100 μg EDTA. The sample was dried down to 0.2 ml and radioactivity was extracted into 1 ml of ethanol-acetone (1:1). After centrifugation for 10 min at 5000 × g, a fraction of the supernatant was applied to the filter paper, and the chromatographic separation of 3H-NE and its metabolites was performed using a method described by Vogt (11) and Langer (12).

Since 4-hydroxy-3-methoxy mandelic acid (VMA) and 3,4 dihydroxy phenyl glycol (DOPEG) have the same Rf value in the phenol with 0.5% 0.1 N HCl (v/w) chromatogram separation, adequate separation between VMA and DOPEG was achieved with phenol-HCl: n-butanol: acetic acid (2:2:1) on the non-washed chromatographic filter paper.

For determination of radioactivity, the chromatogram was divided into 1 cm strips from the origin to the solvent front. Each strip of paper was placed in a glass vial containing 10 ml of Bray solution and counted. Quenching was corrected by the addition of tritiated water standard in this experiment.

**Norepinephrine content**

The aortic strips were incubated in a norepinephrine Ringer medium in the absence or presence of the drug pretreatment for 1 hr and tissue norepinephrine content was measured by the trihydroxyindole method. The thoracic aortae were first blotted on filter paper, weighed, minced and homogenized in ice cold 0.4 N perchloric acid. The homogenate was then centrifuged at 10,000 × g for 10 min, the supernatant adjusted to pH 4.0 with NaOH, and norepinephrine in the extract absorbed on a Dowex 50W-X4 200 to 400 mesh of 6 cm height in the 6 mm diameter columns for elution with 8 ml of 1 N hydrochloric acid (13). The trihydroxy indole reaction was performed with ferricyanide.

The Aminco-Bowman spectrophotofluorometer was used to measure fluorescence at the activation and fluorescence wavelength of 400-504 nm for norepinephrine. The recovery of a known amount of norepinephrine by our technique was about 92%. In these series of experiments, the tissue norepinephrine content was expressed as μg/g of tissue. In several experiments, the aortae used were obtained from rabbits which had been given 6-hydroxydopamine (6-OHDA, 50 mg/kg, i.v. Regis Lab.) in saline solution containing 1 mg/ml ascorbic acid, 24 hr prior to sacrifice.

**RESULTS**

**Uptake and retention of 3H-norepinephrine**

The effects of cold storage and of cocaine on tissue 3H-NE uptake depended to some extent on the loading concentration of 3H-NE in the incubation medium.

When the aortic strips were exposed to low concentrations of 3H-NE (10⁻⁷ M) the cold storage treatment progressively reduced the 3H-NE uptake by the tissue (Fig. 1). On
the 7th day of cold storage, the uptake was found to have remained constant and did not decrease further. Similarly, addition of cocaine (10^-5 M) to the medium in which the tissue was preincubated for 20 min reduced 3H-NE uptake of the fresh tissue by about 64%.

In the tissue stored for 5 days, cocaine failed to inhibit the residual 3H-NE uptake. Desipramine (10^-6 M), which also inhibits norepinephrine uptake by the nerve endings, produced effects on 3H-NE uptake which were similar to cocaine. The washout procedure revealed that the retention of 3H-NE also progressively decreased with prolonged cold storage (Table 1). The level of 3H-NE retention in tissues after 5 days of cold storage remained stable and was not affected by cocaine treatment. After cooling the bath temp.

**Table 1. Effect of cold storage and cocaine on the residual radioactivity after washout of 3H-NE in rabbit aortae.**

<table>
<thead>
<tr>
<th>Duration of cold storage (days)</th>
<th>No. of exps.</th>
<th>Control 3H-NE (ml/g)b</th>
<th>Cocaine (10^-5M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>2.50±0.13</td>
<td>0.60±0.10^4</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>1.43±0.15^c</td>
<td>0.70±0.09^d</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>1.10±0.15^e</td>
<td>0.88±0.09</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>0.91±0.09^f</td>
<td>0.78±0.14</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>0.90±0.13^g</td>
<td>0.83±0.10</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>0.85±0.11^h</td>
<td>0.84±0.13</td>
</tr>
</tbody>
</table>

a Tissues were exposed to l-3H-NE (10^-7 M) for 10 min, followed by washout with fresh medium for 10 min.
b Tissue/medium ratio.
c Significantly different from the control value of fresh tissue (without cocaine treatment) (P<0.001).
d Significantly different from corresponding control values (without cocaine treatment) (P<0.001).

**Fig. 1.** Effect of cold storage on 3H-NE uptake (10^-7 M) by rabbit aortic tissues in the absence (○--○) or presence (△--△) of cocaine (10^-5 M). Tissue was exposed to 10^-7 M l-3H-NE for 10 min. Dotted line indicates the 3H-sorbitol uptake by tissue. Filled circles (●) indicate desipramine (10^-6 M) treatment.
to 2°C, the level of norepinephrine uptake by fresh tissue was reduced, and the uptake level (1.21±0.03 tissue/medium ratio, n=7) was not significantly different from that of the cocaine-treated preparation.

After 6-OHDA treatment, the uptake of 3H-NE at a low loading concentration (10⁻⁷ M) by tissues was reduced by approx. 70%. Cocaine treatment had no effect on the uptake of the 6-OHDA treated tissue (Table 2). The residual norepinephrine uptake after 6-OHDA treatment was also not affected by cold storage treatment for 5 days (1.0±0.1 tissue/medium ratio, n=7).

When tissues had been incubated in a medium containing high 3H-NE (10⁻⁴ M) con-
centration, even after 15 days of cold storage, the rate of $^3$H-NE uptake was the same as that of fresh tissues. This uptake was not significantly affected by cocaine or metaraminol (both $10^{-5}$ M) (Fig. 2). However, the application of phenoxybenzamine ($10^{-5}$ M) decreased this uptake by approx. 24% in both fresh and stored tissues (Table 2). In the presence of metaraminol ($10^{-5}$ M), which inhibits neuronal uptake, phenoxybenzamine caused similar inhibitory effects on the uptake of $^3$H-NE.

To measure the tritium level in the extracellular fluid space, tissues were exposed to $^3$H-sorbitol ($10^{-7}$ M) for 10 min and the tissue radioactivity was determined. There was no significant difference in $^3$H-sorbitol distribution among fresh, cold stored, or 6-OHDA-treated tissues (Table 2).

$^3$H-NE uptake by old rabbit aortae

After exposure to low concentrations of $^3$H-NE the total noradrenaline uptake by older rabbit aortae was significantly less than that observed in young rabbit aortae (Table 2).

![Graph](image-url)

**Fig. 3.** Norepinephrine uptake by fresh and cold stored aortae during the incubation with $^3$H-NE ($10^{-7}$M) and the washout of the accumulated, labeled norepinephrine during the washing with amine-free medium.

- ○○ fresh preparation
- △-△ fresh tissue with cocaine ($10^{-5}$ M)
- □-□ $^3$H-sorbitol uptake by fresh tissue
- ●● cold stored (7 days) preparation
- ▲-▲ cold stored tissue with cocaine ($10^{-5}$ M)
- ■-■ $^3$H-sorbitol uptake by cold stored tissue
After cocaine (10⁻¹¹ M) treatment, however, the uptake of ³H-NE by both young and older rabbit aortae was the same. Thus, the cocaine sensitive norepinephrine uptake in older rabbit aortae was approx. 55% of the value of young rabbit aortae (Table 2).

In ³H-sorbitol incubated tissues, there was no difference in the ³H-sorbitol content between young and older rabbit aortae. Similarly, the uptake of ³H-NE by the aortae of both young and older rabbits at high loading concentrations (10⁻³ M) was not significantly different (Table 2).

Retention curve of ³H-NE

Fig. 3 indicates the retention curve (after washout) of ³H-NE and ³H-sorbitol and also the time course of ³H-NE uptake by the tissues in fresh, cold stored (7 days) and cocaine-treated aortae. The radioactivity of ³H-NE of the fresh and cold stored tissues progressively decreased and almost reached a constant level in 4 to 5 min. The rate of decline of the ³H-NE retention curve in fresh tissues paralleled the curve obtained from cold stored tissues. The retention curve of ³H-NE in the cold stored tissues was not significantly different from the curve of the fresh or cold stored, cocaine-treated tissues (Fig. 3).

Analysis of ³H-norepinephrine and its metabolites

After a 10 min incubation in ³H-NE, ³H-NE constituted nearly 90% of the total radioactivity in both fresh and cold stored tissue (Table 3). It should be noted however, that the total radioactivity of fresh tissue was approx. 2.3 times greater than that of cold stored tissue. The other portion of radioactivity in both tissues was normetanephrine (NMN), which was formed through the action of catechol-0-methyltransferase (COMT) alone, and 4-hydroxy-3-methyltransphenylglycol (MOPEG) (except for cold stored tissue) and 4-hydroxy-3-methoxymandelic acid (VMA), which are deaminated-0-methylated products. Deaminated products such as 2,4-deoxymandelic acid (DOMA) and 3,4-dihydroxyphenylglycol (DOPEG) in both tissues were virtually unmeasurable. The radioactivity (cpm) of VMA, NMN and MOPEG was greater in fresh than in cold stored tissues. The radioactivity, present in medium which had been in contact with fresh tissue, was mainly

| Table 3. Effect of cold storage on the radioactive compounds from rabbit aorta and in the incubation medium. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment       | Specific radioactivity cpm/mg or µl cpm (× 10²) | Total radioactivity | % Total radioactivity |
|                 | NE | DOMA | DOPEG | VMA | NMN | MOPEG |
| Fresh Tissue    | 1806 | 381 | 88.5 | 0.5 | - | 2.5 | 7.4 | 1.6 |
| Medium          | 559 | 1158 | 89.2 | - | - | 2.6 | 3.3 | 4.8 |
| 7 days of cold  | 828 | 166 | 86.0 | - | - | 3.3 | 10.7 | - |
| storage Medium  | 665 | 1446 | 91.3 | - | - | 2.0 | 4.8 | 2.0 |

a Tissue (200 mg) was incubated in medium (2 ml) containing ³H-NE (10⁻⁵ M).
b Radioactivity in the medium which have been in contact with tissue for 10 min.
c Not detectable.
Note: each value is the mean of 3 experiments.
in the form of unaltered $^3$H-NE (89.2%). Similar results were obtained from the incubation medium of cold stored tissue. Radioactivity was not detectable (in either incubation medium) in the portions corresponding to RF value of DOMA and DOPEG, even when using phenol-hydrochloric acid or acid-butanol-acetic acid systems. The NMN content of medium in contact with cold stored tissue was slightly greater than that in the incubation medium for fresh tissue. On the other hand, the levels of total deaminated-0-methylated products (MOPEG plus VMA) in the incubation medium for cold stored tissue were less than those in medium for fresh tissue.

These results probably indicate that enzyme activity of monoamine oxidase (MAO) is more sensitive to cold storage than COMT.

**Tissue norepinephrine content**

After incubation in the low norepinephrine ($10^{-6}$ M) medium, the norepinephrine content of fresh tissues was increased about two-fold over the pre-incubation control level, indicating a clearance of norepinephrine by the tissue from the medium. (Fig. 4). However, the tissue norepinephrine content of cold stored preparations (more than 4 days) did not increase, indicating an inhibition of norepinephrine removal by the tissue. Cocaine ($10^{-3}$ M) treatment also blocked this increase of norepinephrine content in the fresh tissue; however it had no effect on the tissue norepinephrine content of the cold stored tissue (Fig. 4).

When aortic tissues were incubated in the medium containing a high norepinephrine concentration ($10^{-6}$ M), the norepinephrine content of fresh preparations was increased

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**Fig. 4.** Effect of cold storage on norepinephrine removal by rabbit aortae from medium containing norepinephrine at different concentrations. One of the tissue groups was incubated in the medium with high norepinephrine concentration ($10^{-3}$ M) in the absence (●—●) or presence (▲—▲) of cocaine ($10^{-6}$ M). Other groups were incubated in the medium with low norepinephrine concentration ($10^{-6}$ M) in the absence (○—○) or presence (△—△) of cocaine ($10^{-3}$ M). Thin line (■—■) indicates the endogenous norepinephrine content of the tissue before and after cold storage.
about 5-6 fold. Prolonged cold storage now did not inhibit the increase in tissue norepinephrine content, nor did cocaine pretreatment have any effect on the elevated tissue norepinephrine content of either fresh or cold stored tissues (Fig. 4). Phenoxylbenzamine (10^-6 M) treatment, however, reduced the elevation of norepinephrine content by approx. 65% (10 experiments).

The endogenous norepinephrine content was decreased to one-third the original level after a 6-day cold storage.

**DISCUSSION**

It is generally accepted that cocaine potentiation of norepinephrine arises as a result of inhibition of its uptake by the adrenergic nerve terminal, thus making available more norepinephrine at effector sites (1, 14, 15, 16, 17, 18). This concept is also supported by the observation that cocaine does not potentiate the effect of certain sympathetic amines in the sympathetically denervated tissues. (19-23). However, Shibata et al., (24) indicated that chemical sympathectomy with 6-OHDA induced prejunctional supersensitivity as well as postjunctional supersensitivity in rabbit aortae. Furthermore, the experiments on the cold stored aortic strips point to an additional factor which contributes to cocaine potentiation (5, 7, 9).

Previously, Varma and McCullogh (5) observed a decrease in the accumulation (uptake) of 3H-NE by aortic tissue after cold storage. Based on the critique by Graefe and Trendelenburg (10), these data only indicate a measurement of retention and not uptake of 3H-NE. Thus, the question is whether or not cold storage of aortic strips leads to significant inhibition of norepinephrine uptake by adrenergic nerves, the presumed cocaine-sensitive sites of norepinephrine uptake. The results of present experiments clearly indicate that cold storage inhibits the uptake of 3H-NE by aortic tissues. Moreover, cocaine blocks norepinephrine uptake in fresh preparations, but does not affect the residual uptake in the cold stored tissues. If cocaine acts only to inhibit neuronal uptake of catecholamines (14, 25, 26), our findings would suggest that cold storage treatment similarly impairs the uptake mechanism of nerve terminals in vascular smooth muscle. This is supported by the fact that the uptake of cold stored (more than 5 days) tissues was about the same as that observed in the cocaine or 6-OHDA treated preparations.

Even in cold stored aortic strips devoid of neuronal norepinephrine uptake activity, cocaine still potentiates the norepinephrine response (5, 7, 9). Thus, there is an apparent lack of correlation between the blockade of norepinephrine uptake by cocaine and its ability to potentiate norepinephrine action on the vascular smooth muscle. This result strongly supports our previous proposal that cold storage causes functional disruption of rabbit aortae nerve terminals rather than a reserpine-like action without impairment of catecholamine uptake activity (7). This also agrees with the suggestion that prolonged cold storage produces degeneration of the isolated nerve tissues in smooth muscles (1, 27, 28).

Since accumulation of exogenous norepinephrine occurs only when both neuronal uptake and vesicular storage are intact, a failure of the tissue to accumulate 3H-NE is not
necessarily indicative of a failure of the uptake across the nerve membrane. Thus, to measure the total uptake, one should determine also the portion of \(^3\)H-NE which has been metabolized and may be present in the medium. If storage is affected while uptake is not, this would be reflected in less \(^3\)H-NE in the tissue but more \(^3\)H-NE metabolites in the medium. However, this possibility is ruled out by the present finding that the major portion of the total radioactivity in the fresh and cold stored tissues was \(^3\)H-NE, and not its metabolites.

In addition, the amount of \(^3\)H-metabolites which were formed by monoamine oxidase in the incubation medium for the cold stored tissue was less than that in the medium for the fresh tissue. Moreover, both fresh and stored strips lost their radioactivity proportionately as a result of the washout (Fig. 3). Thus it is unlikely that cold storage selectively inhibits the intraneural binding of norepinephrine. It is concluded that cold storage treatment inhibited the inward transport of norepinephrine across the neuronal membrane.

The decrease in \(^3\)H-NE uptake at low temp. leads to the speculation that the uptake process is under specific energy requirement. In this regard, Nedergaard and Bevan (29) also reported that a lowering of bath temp. causes a linear decrease in \(^3\)H-NE uptake by rabbit aortae and that metabolic inhibitors (DNP, sodium cyanide, and isoiodoacetic acid) partially inhibit the \(^3\)H-NE uptake, which supports the possibility that part of the uptake is by a carrier-mediated transport mechanism.

When low norepinephrine concentration (10\(^{-5}\) M) medium was used, cold storage or cocaine treatment markedly decreased the norepinephrine removal of aortic tissues. Cocaine treatment did not add further to the inhibitory effect caused by the cold storage procedure. These data also suggest that cold storage treatment severely impairs the ability of the nerve terminals to take up exogenous catecholamines, and excludes the possibility that a significant portion of the labeled amine had entered by displacement of endogenous catecholamines from bound sites.

Trendelenburg et al. (30) suggested that cocaine potentiation is greatest in tissues with high density of adrenergic innervation such as in the cat nictitating membrane and least in tissues sparsely innervated, as in aortic smooth muscles. However, Shibata et al. (7) found that although young rabbit aortae have a higher density of adrenergic innervation than older rabbit aortae, cocaine potentiates the norepinephrine response of both strips to the same extent in spite of the significantly greater \(^3\)H-NE uptake and cocaine blockade of uptake in the young aortae. Therefore, these results cannot be explained solely by the presumed mechanism of cocaine potentiation of norepinephrine through inhibition of uptake.

Histochemical studies suggest that extraneuronal uptake of norepinephrine also occurs in vascular and other smooth muscles (31–33). This process corresponds to norepinephrine uptake by isolated tissues at high concentrations which is blocked by phenoxybenzamine but not by cocaine (34, 35). The present experiments indicate that cold storage has no effect on norepinephrine uptake at the high loading concentration (10\(^{-5}\) M) and phenoxybenzamine reduces this norepinephrine uptake by the fresh and cold stored tissues, whereas
cocaine does not. Furthermore, cold storage and cocaine have no effect on the removal of norepinephrine by the tissue when the tissue is incubated in a medium containing a high norepinephrine concentration \(10^{-5} \text{ M}\). It is apparent that there is extraneuronal uptake and this process is resistant to cold storage treatment.

Histochemical studies indicate that phenoxybenzamine does not prevent norepinephrine from binding to collagen and elastic tissue of blood vessels (33). It appears likely that the aortic smooth muscle cell is mainly responsible for the phenoxybenzamine sensitive extraneuronal uptake of norepinephrine in both fresh and cold stored preparations. The phenoxybenzamine \(10^{-5} \text{ M}\) resistant norepinephrine uptake then may be accounted for by the binding in connective tissues.

In conclusion, cold storage treatment selectively inhibits the cocaine sensitive neuronal uptake of norepinephrine, and the responses of cold stored aortic tissues to norepinephrine are potentiated by cocaine (7). Therefore, the development of cocaine-supersensitivity cannot be solely explained by inhibition of neuronal uptake; cocaine potentiation also has a post-synaptic component.

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