Effect of Various Pretreatments on Responses to Ephedrine Isomers

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Tachyphylactic tendencies of the ephedrine isomers and their interaction with cocaine, methylphenidate, pipradrol, D(−)pseudoephedrine, and guanethidine have been studied in anesthetized, vagotomized, atropinized dogs. Blood pressure and heart rate were recorded. Among the three pressor isomers, D(−)ephedrine was shown to be the isomer most resistant to the development of tachyphylaxis, L(+)pseudoephedrine was shown to have the greatest tendency, while L(+)ephedrine was the isomer with a moderate tendency to tachyphylaxis. Thirty-minute pretreatment with either cocaine, methylphenidate, pipradrol or D(−)pseudoephedrine or 24-hr. pretreatment with guanethidine was observed to produce a surmountable antagonism to the pressor activity of D(−)ephedrine. Similar pretreatment resulted in an insurmountable antagonism to the pressor activity of L(+)ephedrine and L(+)pseudoephedrine; in fact, depressor activity was observed with these isomers. With some exceptions, the pretreatments resulted in parallel antagonisms in the chronotropic effects of the ephedrine isomers.

DIFFERENTIAL sympathomimetic amine antagonism recently has been the source of extensive research. Cocaine (1, 2), methylphenidate (3, 4), pipradrol (5), guanethidine (6–8), and D(−)pseudoephedrine (9, 10) have been shown to affect the responses to sympathomimetic amines, so that direct acting amines are augmented, amines with both direct and indirect components are surmountably antagonized, while indirect acting amines are insurmountably antagonized. Ephedrine has been shown to fall in the group of compounds which have both a direct and indirect action.

Ephedrine has two asymmetric carbon atoms and therefore can exist as four possible isomers. Close (11) investigated the configuration of the ephedrine isomers and established pseudoephedrine as being threo and ephedrine as being erythro with respect to the amine and hydroxyl groups. Chen *et al.* (12) studied the pressor effects of these isomers in the spinal cat and reported the following order of pressor activity: D(−)ephedrine > L(+)ephedrine > L(+)pseudoephedrine > D(−)pseudoephedrine. Similar results in the anesthetized dog were obtained by Patil (10), except that D(−)pseudoephedrine showed only depressor activity. Patil also demonstrated that pretreatment with reserpine will abolish the pressor activity of L(+)ephedrine and L(+)pseudoephedrine. This evidence strongly suggests that their pressor activity resides in an ability to release endogenous catecholamines. Therefore, ephedrine presents an opportunity to study direct and indirect acting compounds within the same basic entity; the direct acting isomer is D(−)ephedrine, while L(+)ephedrine and L(+)pseudoephedrine represent indirect acting compounds.

The results reported herein represent a study of tachyphylactic tendencies of the ephedrine isomers and their antagonism by cocaine, methylphenidate, pipradrol, guanethidine, and D(−)-pseudoephedrine.

EXPERIMENTAL

Adult mongrel dogs of either sex, weighing from 6.8 to 14.0 Kg., were used as the experimental animals. After surgical anesthesia was induced with sodium thiopental, 15 mg./Kg. i.v., and sodium barbital, 250 mg./Kg. i.v., atropine, 1 mg./Kg. i.v., was administered. In addition, bilateral vagotomy was performed. Following tracheotomy, the right carotid artery was cannulated and the blood pressure recorded *via* a mercury manometer on a kymograph. The right femoral vein was cannulated for the injection of drug solutions. The heart rate was recorded on a recorder (Sanborn Twin Viso, model 62). Three to five animals represent each observation.

The isomers of ephedrine were prepared by the method described by LaPidus *et al.* (9), and drug solutions of the isomers were made in physiological saline with the aid of dilute hydrochloric acid. The pH of these solutions was always within physiological limits. For each experiment, fresh drug solutions were prepared. The doses of the ephedrine isomers used in this experimentation were established by the work of Patil (10).

Solutions of the antagonists, cocaine, D(−)pseudoephedrine, methylphenidate, and guanethidine, were prepared as $^1/_5$ *M* to the free base in physiological saline. Solutions of the antagonist, pipradrol, due to solubility limitations, were not obtainable as $^1/_5$ *M* to the free base; therefore, a stock solution containing 15 mg./ml. of the free base was prepared in physiological saline. All doses of the experimental drugs (isomers and antagonists) refer to milligrams per kilogram of the free base.

All antagonists, except for pipradrol, were administered on the basis of 0.2 ml./Kg. of $^1/_5$ *M* solution. In pipradrol, the dosage was figured in the

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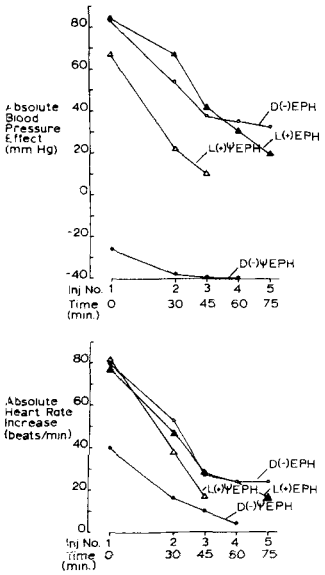

Fig. 1.—Tachyphylactic tendencies of D(−)-ephedrine, 0.33 mg./Kg.; L-(+)ephedrine, 0.99 mg./Kg.; L(+)pseudoephedrine, 1.65 mg./Kg.; and D(−)pseudoephedrine, 3.3 mg./Kg. Each point represents the average blood pressure rise and average heart rate increase of three to five animals.

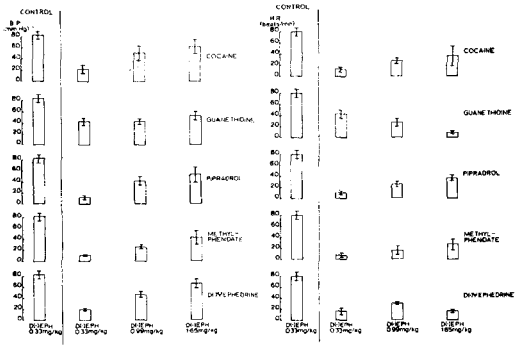

Fig. 3.—The effect on blood pressure and heart rate response in the anesthetized dog to D(-)ephedrine after pretreatment with cocaine, guanethidine, pipradrol, methylphenidate, or D(-)pseudoephedrine. Half-hour intervals between successive doses of D(-)ephedrine. The values represent the average response of three to five dogs. Vertical bars indicate standard deviation.

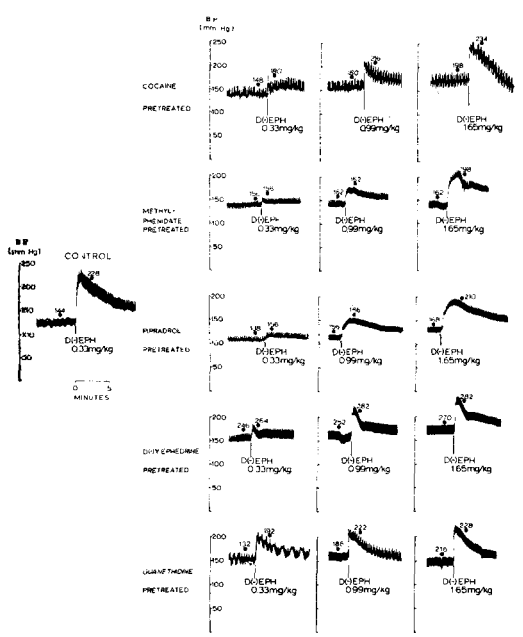

Fig. 2.—Typical surmountable antagonism to the blood pressure response in the anesthetized dog to D(-)ephedrine. Half-hour intervals between successive doses of D(-)ephedrine after any given antagonist. Heart rate indicated by numbers above blood pressure tracings (beats/minute).

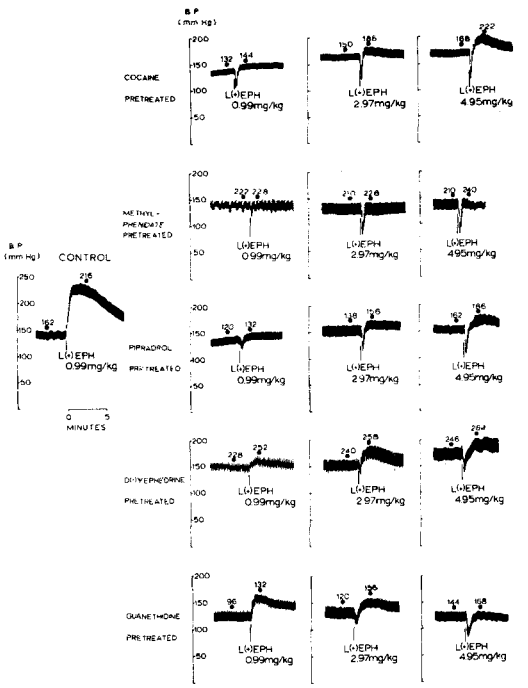

Fig. 4.—Typical insurmountable antagonism to the blood pressure response in the anesthetized dog to L(+)ephedrine. Half-hour intervals between successive doses of L(+)ephedrine after any given antagonist. Heart rate indicated by numbers above blood pressure tracings (beats/minute).

same manner, then the appropriate number of milligrams were obtained from the stock solution. The resulting milligram per kilogram dose levels of the antagonists are within the values reported in the literature.

In the study of tachyphylactic tendencies, the following injection schedule was utilized. An initial injection of a given isomer was administered; 30 min. later a second injection was given. Thereafter, every 15 min. an injection was given until tachyphylaxis was observed. The dose of each individual isomer used in the study of tachyphylaxis was used as the initial challenging dose under antagonism and hereafter is referred to as the control dose.

The antagonists were administered according to the following schedule: cocaine, 30-min. pretreatment (given by slow manual infusion); pipradrol, 30-min. pretreatment; methylphenidate, 30-min. pretreatment; D(−)pseudoephedrine, 30-min. pretreatment; and guanethidine, 24-hr. pretreatment. After the animals were pretreated for the reported time period, they were challenged with the control dose of a respective isomer. One-half hour later,

Fig. 5.—The effect on blood pressure and heart rate response in the anesthetized dog to L(+)-ephedrine after pretreatment with cocaine, guanethidine, pipradrol, methylphenidate, or D(−)-pseudoephedrine. Half-hour intervals between successive doses of L(+)ephedrine. The values represent the average response of three to five dogs. Vertical bars indicate standard deviation.

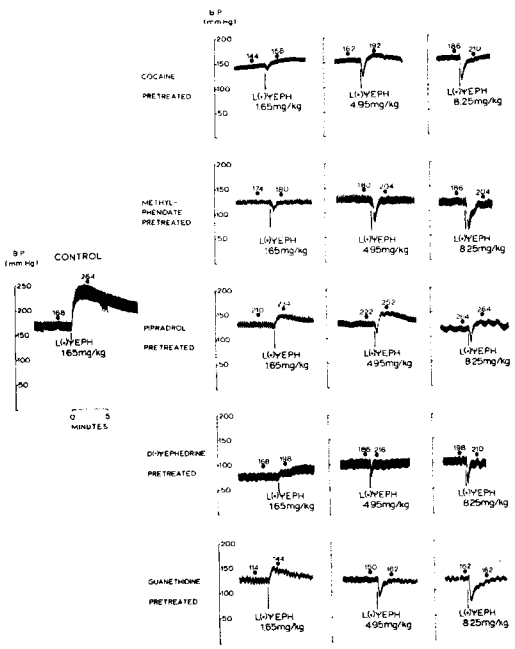

Fig. 6.—Typical insurmountable antagonism to the blood pressure response in the anesthetized dog to L(+)pseudoephedrine. Half-hour intervals between successive doses of L(+)pseudoephedrine after any given antagonist. Heart rate indicated by numbers above blood pressure tracings (beats/minute).

they were challenged again with three times the control dose level and again 0.5 hr. later with five times the control dose level.

In the study of tachyphylaxis, any given animal received only one isomer; and in the studies of antagonism, any given animal received only one antagonist and thereafter only one isomer.

The following drugs were used in the experimentation: D(−)ephedrine, L(+)ephedrine, L(+)pseudoephedrine, D(−)pseudoephedrine, cocaine hydrochloride, pipradrol hydrochloride, methylphenidate hydrochloride, and guanethidine sulfate.

RESULTS

The tachyphylactic tendencies of the ephedrine isomers are represented in Fig. 1. It may be seen that D(−)ephedrine is the isomer most resistant to the development of tachyphylaxis, and L(+)pseudoephedrine shows the greatest tendency for tachyphylaxis. While the difference between the results for L(+)ephedrine and those of D(−)ephedrine are statistically insignificant, it would appear that L(+)ephedrine displays an intermediate tendency for the development of tachyphylaxis. Upon repeated doses of D(−)pseudoephedrine, an increase in depressor action was observed; however, the magnitude of the depressor action of the fourth injection is statistically insignificant from that observed at the initial injection.

Surmountable antagonism to the pressor effect of D(−)ephedrine was observed after each pretreatment (Fig. 2). Similar surmountable antagonism to the chronotropic activity of D(−)ephedrine was observed, except in those animals pretreated with guanethidine (Fig. 3). In guanethidine pretreated animals, increasing doses of D(−)ephedrine resulted in a decreasing chronotropic effect.

Insurmountable antagonism to the blood pressure effect of (L+)ephedrine was observed after each pretreatment (Fig. 4). In animals pretreated with guanethidine, the control dose of L(+)ephedrine was observed to produce a pressor response, but with increasing doses of L(+)ephedrine, less pressor and finally depressor activity was demonstrated. A small pressor response to the control dose of L(+)-ephedrine was observed in animals pretreated with D(−)pseudoephedrine; but with an increase in dose, L(+)ephedrine caused only a fall in blood pressure (Fig. 5). Except for guanethidine pretreated animals, the chronotropic effect of L(+)-ephedrine was surmountably antagonized to some extent.

Insurmountable antagonism to the blood pressure effect of L(+)pseudoephedrine was observed after each pretreatment (Fig. 6). In animals pretreated with guanethidine, the control dose of L(+)pseudoephedrine was observed to produce a pressor response, but with an increase in dose, only depressor activity was produced by L(+)pseudoephedrine. A small pressor effect to the control dose of L(+)-pseudoephedrine was demonstrated in animals pretreated with D(−)pseudoephedrine. Only depressor activity was noted, however, with increasing doses of L(+)pseudoephedrine (Fig. 7). Insurmountable antagonism to the chronotropic effect of L(+)pseudoephedrine was observed with each pretreatment.

Preliminary studies indicated that neither cocaine, methylphenidate, pipradrol, nor guanethidine qualitatively altered the depressor response to D(−)pseudoephedrine at its control dose level.

DISCUSSION

That D(−)ephedrine was demonstrated to have the least tendency for the development of tachyphylaxis is in agreement with the results of Patil (10). D(−)Ephedrine has been placed in the group of sympathomimetic amines which possess both direct and indirect components (1, 2). Thus, the reduction in pressor response, which occurred from the initial injection of D(−)ephedrine to the third

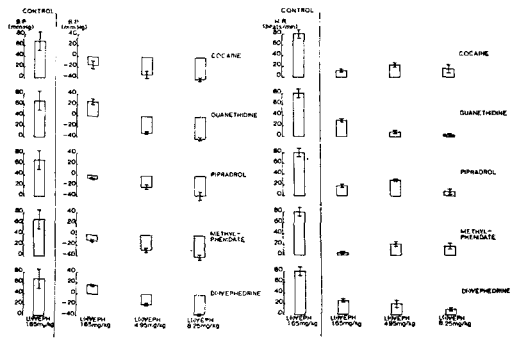

Fig. 7.—The effect on blood pressure and heart rate response in the anesthetized dog to L(+)-pseudoephedrine after pretreatment with cocaine, guanethidine, pipradrol, methylphenidate, or D(−)pseudoephedrine. Half-hour intervals between successive doses of L(+)pseudoephedrine. The values represent the average response of three to five dogs. Vertical bars indicate standard deviation.

injection, would appear to be a loss of the indirect component, the loss of the ability to release endogenous catecholamines. After the loss of the indirect component, only the direct component remains, and a response plateau appears. Patil (10), while studying the tachyphylaxis to the pressor response of D(−)ephedrine, noted the same plateau, which remained constant for many more injections than used in this work.

Since reserpine has the ability to abolish the pressor effects of L(+)ephedrine and L(+)pseudoephedrine (10), the liberation of endogenous catecholamines would seem to be necessary for their activity. Greater tachyphylactic tendencies would therefore be expected than for D(−)ephedrine, which has both direct and indirect components.

The tendency for the depressor response to increase after repeated injections of D(−)pseudoephedrine suggests that D(−)pseudoephedrine, in addition to its intrinsic depressor activity, possesses the ability to liberate some catecholamines. Since the tendency is to increase the depressor response, the effect should not be regarded as tachyphylaxis.

A similar development of tachyphylaxis to the positive chronotropic effect of the isomers was observed, and the above-mentioned explanations can be expanded to include them.

In the present mode of thinking, cocaine's potentiation of injected norepinephrine is a result of inhibition of the uptake of the amine into storage sites in sympathetic effector organs. This permits a higher concentration of the amine for activation of adrenergic receptors (13–16). Cocaine is also believed to antagonize tyramine by an interference with the release of endogenous catecholamines from peripheral storage sites (1, 15, 17). By combining these two ideas, one could infer that cocaine probably acts at the neuron membrane at a common site of entrance or exit of endogenous catecholamines. That cocaine has no depleting action on peripheral catecholamine stores has been demonstrated (5, 18).

The reduced pressor response of D(−)ephedrine after cocaine pretreatment represents a loss of the indirect component of the isomer. Cocaine's interference with the release of endogenous catecholamines leaves only the direct component to elicit the pressor response. With an increase in dosage of D(−)ephedrine, increased direct action is obtained, and the antagonism due to cocaine is surmounted. A similar surmountable antagonism to the chronotropic activity of D(−)ephedrine was observed.

Cocaine not only suppressed the pressor activity of L(+)ephedrine and L(+)pseudoephedrine, but also in cocaine pretreated animals these isomers demonstrated only depressor action. Since the liberation of endogenous catecholamines appears necessary for the responses to L(+)ephedrine and L(+)pseudoephedrine, and cocaine has the ability to prevent this liberation, the results observed would be expected. Cocaine appears able to antagonize all indirect actions of sympathomimetic amines (19); therefore, increase in dose of the L-isomers should not result in a pressor response.

Under cocaine antagonism, L(+)ephedrine did produce some degree of surmountability to its chronotropic activity with increasing dosage. L(+)-Ephedrine may have more direct activity in cardiac tissue than it has on the arterial bed. Trendelenburg (19) suggests that for a given amine the ratio of direct to indirect activity may vary from tissue to tissue. The chronotropic effect of L(+)pseudoephedrine was insurmountably antagonized by cocaine; thus, L(+)pseudoephedrine appears to be mainly indirect in both the arterial bed and cardiac tissue.

Methylphenidate increases both pressor and nictitating membrane responses to norepinephrine and epinephrine, while reducing the responses to tyramine and ephedrine (5). Thus, methylphenidate has the ability to potentiate direct acting compounds while antagonizing the action of indirect compounds.

The reduced pressor activity of D(−)ephedrine after methylphenidate pretreatment represents a loss of the indirect component. With an increase in dose of D(−)ephedrine, increased direct effect accounts for the increased pressor response. A similar surmountable antagonism to the chronotropic activity of D(−)ephedrine was observed, although the extent of recovery was not great.

Methylphenidate antagonized the pressor activity of L(+)ephedrine and L(+)pseudoephedrine, and only a depressor action was observed. Since the literature cited indicates that methylphenidate blocks the liberation of catecholamines, the case is similar to that of cocaine antagonism. The L-isomers, prevented from displaying their amine releasing property, could only exhibit depressor activity. The chronotropic activity of the L-isomers was greatly reduced by methylphenidate pretreatment, but some increase in chronotropic action was observed with increasing dosage.

The findings of Farrant (5) that pipradrol can potentiate exogenous catecholamines, while causing subsensitivity to tyramine and ephedrine, indicate that it can separate direct and indirect components of a given compound.

Pipradrol was effective in antagonizing the pressor activity of D(−)ephedrine by the elimination of the indirect component. With increasing dosage of D(−)ephedrine, increased direct action accounts for the increased pressor activity. The chronotropic effect of D(−)ephedrine was similarly antagonized by pipradrol.

The L-isomers were found to show only depressor

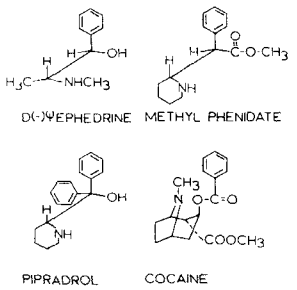

Fig. 8.—Structural similarity between the antagonists D(−)pseudoephedrine, methylphenidate, pipradrol, and cocaine.

effects under pipradrol pretreatment; and with increasing doses of these isomers, only increased depressor activity was noted. Both L-isomers retained some chronotropic action after pipradrol. L(+)Ephedrine was able to exhibit greater increases in heart rate effects with increasing dosage than was L(+)pseudoephedrine.

D(−)Pseudoephedrine has been shown to potentiate the direct acting amines, norepinephrine and epinephrine, and to block the pressor and heart rate effect of the indirect acting compound, amphetamine (10). Therefore, it has the ability, common to the other antagonists, to separate direct and indirect actions of a compound.

D(−)Pseudoephedrine was found to reduce the pressor effect of D(−)ephedrine by the elimination of the indirect component, leaving only the direct action component. This antagonism was surmounted by an increase in dosage of D(−)ephedrine. A similar antagonism to the chronotropic effect of D(−)ephedrine was encountered with D(−)pseudoephedrine pretreatment. Increasing the dose of D(−)ephedrine threefold did result in partial restoration of chronotropic activity, but a fivefold increase in dose resulted in less chronotropic activity than did the threefold increase in dose. This reduction in chronotropic action of D(−)ephedrine at the fivefold increase in dosage may be due to minor blockade of the direct receptor site in addition to the indirect site blockade.

Both L-isomers retained some pressor activity at their respective dose levels in D(−)pseudoephedrine pretreated animals. If incomplete blockade of the indirect receptor site occurred, then a small amount of endogenous catecholamines would be available for liberation—hence the pressor effect. This amount of available endogenous amines could be liberated by the initial dose of the L-isomers, and further injections would now be ineffective in bringing about a pressor response. D(−)Pseudoephedrine was effective in producing an insurmountable antagonism to the chronotropic effects of the L-isomers.

Guanethidine has been reported by various workers to cause depletion of amine stores in various tissues (20–23). Twenty-four-hour pretreatment with guanethidine was observed to depress the pressor response of D(−)ephedrine. This reduction of the pressor response is attributed to the loss of indirect activity through depletion of catecholamine stores. Increasing the dosage of D(−)ephedrine did regain some further pressor effect, although the extent of recovery was not great.

Increasing doses of D(−)ephedrine produced decreasing chronotropic effect in guanethidine pretreated animals. The mechanism of this effect is obscure. If most of the chronotropic effect of D(−)-ephedrine is indirect in nature, then the observation could be explained by incomplete depletion of catecholamine stores by guanethidine. To explain the results of the chronotropic activity of D(−)ephedrine under the other antagonists, we could point out that cocaine, methylphenidate, pipradrol, and D(−)-pseudoephedrine do potentiate catecholamines. Such an explanation, however, is not in keeping with the fact that all of the antagonists have the ability to abolish indirect activity, nor with the results of the study of tachyphylactic tendency of D(−)ephedrine. If D(−)ephedrine's cardiac effect was indirect in nature, then we would expect a tendency to develop tachyphylaxis similar to that of the L-isomers. The observed plateau effect in the cardiac activity of D(−)ephedrine suggests a direct component.

Incomplete depletion of amine stores could explain the observed pressor and heart rate activity of the L-isomers in animals pretreated with guanethidine.

Since similar antagonistic effects were encountered with cocaine, methylphenidate, pipradrol, and D(−)pseudoephedrine, the suggestion is made that they may be acting in a similar manner at indirect receptor sites. Each of these antagonists can exist in a configuration in which an aromatic center, an oxygen function (ester or hydroxyl), and an amine function occur in a manner similar to that encountered in many sympathomimetic amines. If we accept the hypothesis (24) that three groups in an optically active drug are concerned with its attachment to tissue receptors, these antagonists could attach themselves firmly to receptors and prevent the ephedrine isomers from combining with the indirect receptor sites. Figure 8 depicts similar configurations in which cocaine, methylphenidate, pipradrol, and D(−)pseudoephedrine can exist. Guanethidine cannot exist in a similar form, and its antagonism is attributed to a depletion of catecholamine stores.

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