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THE ACTION OF TYRAMINE ON THE DOG ISOLATED ATRIUM

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It has been confirmed that tyramine has positive inotropic and chronotropic actions on the dog isolated atrium. These responses were incompletely and reversibly inhibited by cocaine, but completely and irreversibly blocked by phenoxybenzamine. Blockade of the atrial β-receptors by dichloroisoprenaline could be overcome by noradrenaline and by larger doses of tyramine. With the aortic strip of the reserpinized rabbit for assay, tyramine was shown to release a vasoactive material from the dog atrium whose receptors were blocked by dichloroisoprenaline. The use of an anti-histamine and an anti-5-hydroxytryptamine agent (cyproheptadine) appeared to exclude the possibility that the effect was due to the release of histamine or 5-hydroxy-tryptamine from the atrium by tyramine. Further observations of the action of the vasoactive material on the guinea-pig ileum and on the rat fundal strip strongly suggested that the material was a catechol amine. It was concluded that under these conditions tyramine acts by liberating catechol amines from storage sites so that the amines are free to act at receptor sites. The behaviour of the atrium to tyramine in the presence of cocaine or of phenoxybenzamine suggests that the liberation of catechol amines by tyramine differs from the release due to adrenergic nerve stimulation. It is suggested that, after an infusion of tyramine, there is a much slower release of catechol amines than after stimulation of adrenergic nerves.

In 1958, Burn & Rand postulated that tyramine produces sympathomimetic effects by releasing catechol amines from sites where they are bound, so that they are free to act on tissue receptors. Direct evidence to support this view has been sought by various workers with conflicting and often negative results.

Lockett & Eakins (1960) found a great increase in plasma catechol amines from the lower abdominal aorta of the cat after an infusion of tyramine, adrenaline being increased more than noradrenaline. Vane (1960) found no evidence for catechol amine release into the circulation of the cat by tyramine. Weiner, Draskóczy & Burack (1962) showed that tyramine infusions into dogs only occasionally produced slight increases in plasma catechol amines, although this occurred more frequently after treatment with phenoxybenzamine. Burn & Burn (1961) and Hertting, Axelrod & Patrick (1961) have shown that the spontaneous release of [^{3}H]-noradrenaline, taken up by the heart, is increased by tyramine, and Lindmar & Muscholl (1961) demonstrated an increase in the rate of spontaneous release of noradrenaline from the rabbit isolated perfused heart when tyramine was added.

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In the present study an attempt was made to demonstrate the release of endogenous catechol amines, by tyramine, from storage sites in the isolated right atrium of the dog. To facilitate this the atrial β-receptors for catechol amines were blocked with dichloroisoprenaline (Moran & Perkins, 1958). To demonstrate the release of catechol amines from storage sites by tyramine, the fluid bathing the atrium was transferred (after exposing the atrium to tyramine) to another organ-bath containing an aortic spiral strip from a reserpinized rabbit. Controls excluded the possibility that any response of the aortic strip was caused by spontaneous release of vasoactive material from the atrium or by direct action of tyramine in the transferred fluid on the aortic strip itself. It was thought necessary to exclude the active release by tyramine of substances other than catechol amines which could shorten the aortic strip, for example, histamine, 5-hydroxytryptamine and vasoactive polypeptides. The effects of cocaine, dichloroisoprenaline and phenoxybenzamine on the positive inotropic and chronotropic actions of tyramine in the dog isolated atrium were also observed.

METHODS

Isolated right atrium of the dog. Mongrel dogs, 10 to 20 kg in weight, were killed with a captive bolt. The thorax was rapidly opened and the atria were removed and placed in a container filled with oxygenated McEwen's solution at 14 to 15° C. After 20 to 30 min the right atrium was separated from the left atrium and the interatrial septum. The right atrium could be identified by the trabeculated appearance of its muscle fibres, since this arrangement in branching bundles is less obvious in the interatrial septum and in the left atrium. The tip of the right atrial appendage was then attached to a recording lever with thread, and the opposite pole of the atrium to a glass oxygen bubbler in the organ-bath.

Rabbit aortic strip. Rabbits, weighing 1.5 to 2 kg, were given reserpine (1 mg/kg) intraperitoneally once daily for 2 days before they were killed by a blow on the neck. The mediastinum was rapidly removed, and polyethylene tubing was placed in the lumen of the aorta to facilitate the dissection of fat and fascia off the wall. Then, starting at the distal end of the aortic arch and cutting a right-hand spiral downwards with a sharp blade, a strip 0.5 cm wide and 4 cm long was obtained, which gave the most satisfactory results.

Guinea-pig ileum. A piece of ileum, 4 cm long, was taken about 8 cm proximal to the ileocolic sphincter and suspended in the organ-bath.

Rat fundal strip. This was prepared according to the method of Vane (1957).

Organ-bath and solutions. The organ-bath was a thin-walled Perspex bath immersed in a thermostatically controlled water-bath. An overflow ensured that, in the presence of tissue, 25 ml. of bathing fluid was always present.

McEwen's (1956) solution, of the following composition in g/l., was most commonly used: NaCl 7.6, KCl 0.42, $CaCl_2$ 0.24, NaH_2PO_4 0.143, $NaHCO_3$ 2.1, glucose 2.0 and sucrose 4.5.

Tyrode solution and Douglas & Rubin's (1961) modification of Krebs solution were also used. A mixture of 95% O_2 and 5% CO_2 was bubbled through the fluid, giving a *p*H of 7.4. Disodium versenate (2 mg/l.) was added to the bathing fluid to reduce oxidation of added or liberated catechol amines to a minimum (Bevan, 1960). The temperature of the fluid bathing the dog atrium or the guinea-pig ileum was 31° C, and that bathing the aortic spiral and rat fundal strips was 37° C.

Recording system. The amplitude of the atrial contractions was recorded on a smoked drum by a spring lever (Starling type) with a frontal writing point. The atrial frequency was recorded on a Post Office counter, driven by a 120 V dry battery. A copper wire on the lever dipped into a saline trough when the lever moved down and completed an electrical circuit and operated the relay (Huković, 1959). The responses of the other tissues were recorded on a kymograph with an isotonic lever and a frontal writing point.

Transfer experiments. The fluid in the atrial organ-bath (25 ml.) was transferred to another bath containing the tissue for assay, after its temperature had been adjusted to that of the fluid bathing the tissue for assay.

After the atrial β-receptors had been blocked with dichloroisoprenaline (2×10^{-5} for 30 min), tyramine (4×10^{-6}) was added to the atrial preparation for 15 min and the bath fluid was then transferred; this will be called a transfer. Transfer of atrial bath fluid which did not contain tyramine but had been in contact with the atrium for 15 min will be called a blank transfer. As a control tyramine (4×10^{-6}) was added either to the fluid which had been withdrawn from the bath, or directly to the target tissue preparations.

Drugs. The following compounds were used: Noradrenaline bitartrate, tyramine hydrochloride, dichloroisoprenaline (Lilly), phenoxybenzamine (Dibenzyline), cocaine hydrochloride, 5-hydroxytryptamine creatinine sulphate, histamine acid phosphate and cyproheptadine hydrochloride (Merck, Sharp & Dohme).

RESULTS

Viability of the dog isolated atrium. The dog atrium, after an initial settling-down phase, had a regular beat with a frequency of about 50/min (range 36 to 102) and an amplitude of 3 to 6 cm on the kymograph. The atrium was used for at least 8 hr in many of the experiments and probably would have survived much longer if required. Thirty-eight dog atrial preparations were used.

Action of tyramine on the dog atrium. The atrial response to tyramine, like that to noradrenaline, was both inotropic and chronotropic. The atrium gave near maximum responses to noradrenaline (4×10^{-8}) and tyramine (4×10^{-7}). The peak of the noradrenaline response was rapidly reached, but tyramine initially caused a more gradual increase, followed by an abrupt change to the peak effect (Fig. 1). This response could indicate either that added noradrenaline reached the receptors

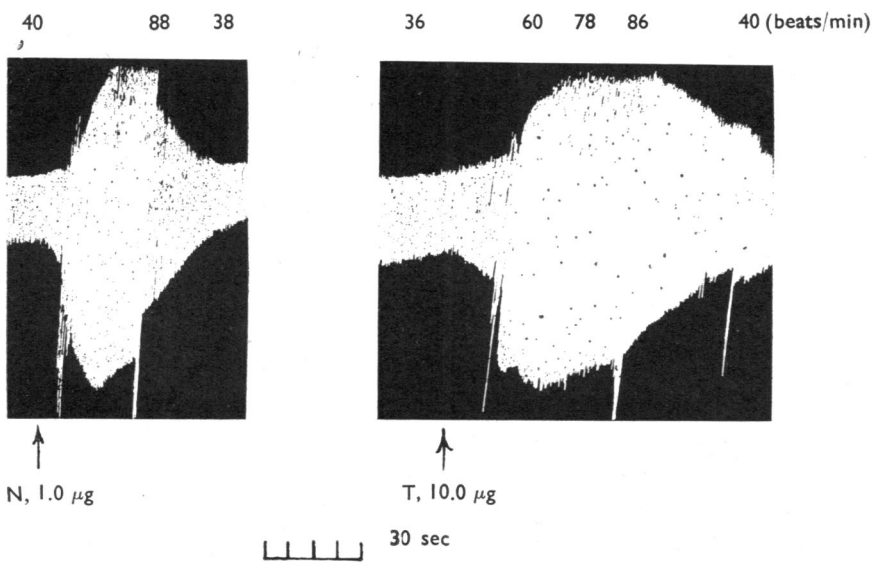

Fig. 1. Contractions of dog atrium; comparison of responses to noradrenaline (N) and tyramine (T). Numerals above records give atrial frequencies (beats/min). Time intervals are 30 sec. In this and subsequent Figs., the doses of drugs were added to a 25-ml. organ bath.

more rapidly than added tyramine, or that tyramine was acting indirectly, as suggested by Burn & Rand (1960).

Effect of cocaine on the atrial response to tyramine. The responses to tyramine (4×10^{-7}) were recorded in three experiments before, and 4 min after, adding cocaine (4×10^{-6}) to the bath (without washing out the bath). There was a great but incomplete inhibition of the atrial response to tyramine. Washing out the cocaine completely restored the response of the atrium to tyramine (Fig. 2).

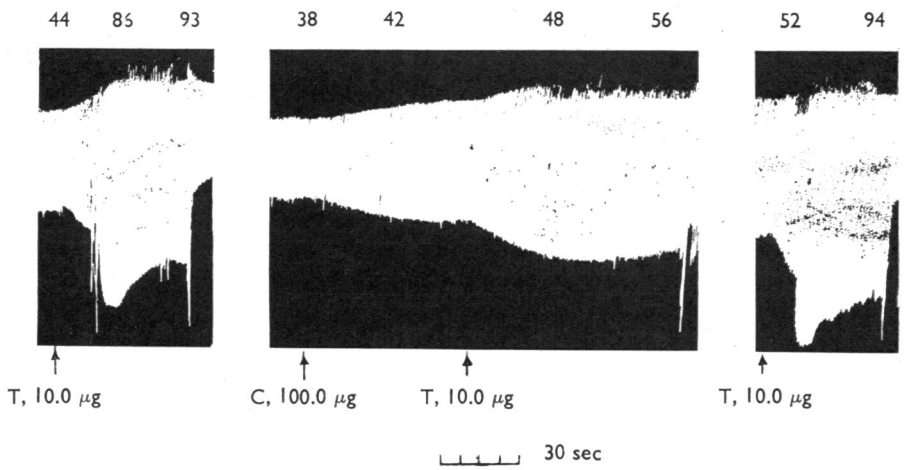

Fig. 2. Contractions of dog atrium; from left, responses to tyramine (T) before, in the presence of, and after cocaine (C). Numerals above the records give atrial frequencies (beats/min). Time intervals are 30 sec.

Effect of dichloroisoprenaline on the response of the dog atrium to noradrenaline and to tyramine. After recording the responses to noradrenaline (4×10^{-8}) and to tyramine (4×10^{-7}) the dog isolated atrium was incubated with dichloroisoprenaline (4×10^{-6}) for 30 min. This blocked the actions of tyramine (4×10^{-7}) and of noradrenaline (4×10^{-8}) on the dog atrium. Higher concentrations of these amines, tyramine (4×10^{-6}) and more definitely noradrenaline (4×10^{-7}), overcame this block.

Effect of phenoxybenzamine on the responses of the dog atrium to added noradrenaline and tyramine. In eleven of fifteen experiments phenoxybenzamine (2×10^{-5}) had positive inotropic and chronotropic actions on the dog atrium which could be repeated early in the experiments but never after 1 to 2 hr.

The potentiation by phenoxybenzamine of the inotropic action of added noradrenaline on rabbit atria, reported by Furchgott (1960), was confirmed in three experiments; the potentiation was three-fold (noradrenaline concentration, 4×10^{-8}). The responses of the dog isolated atrium to noradrenaline (4×10^{-8}) immediately before and 1 to 2 hr after exposing the atrium to phenoxybenzamine (2×10^{-5}) for 30 min were nearly the same; the chronotropic response was unaffected, but in three experiments the positive inotropic response was slightly potentiated.

The effect of phenoxybenzamine (2×10^{-5}) on the response of the dog atrium to tyramine (4×10^{-7}) was observed. Phenoxybenzamine was added to the tissue-bath and 15 min later tyramine produced apparently unaltered inotropic and chronotropic responses. The atrium was exposed to this concentration of phenoxybenzamine for up to 50 min, and 1 hr after washing out the phenoxybenzamine there was no response to added tyramine (4×10^{-7} or 4×10^{-6}) (Fig. 3). This occurred consistently in fifteen experiments.

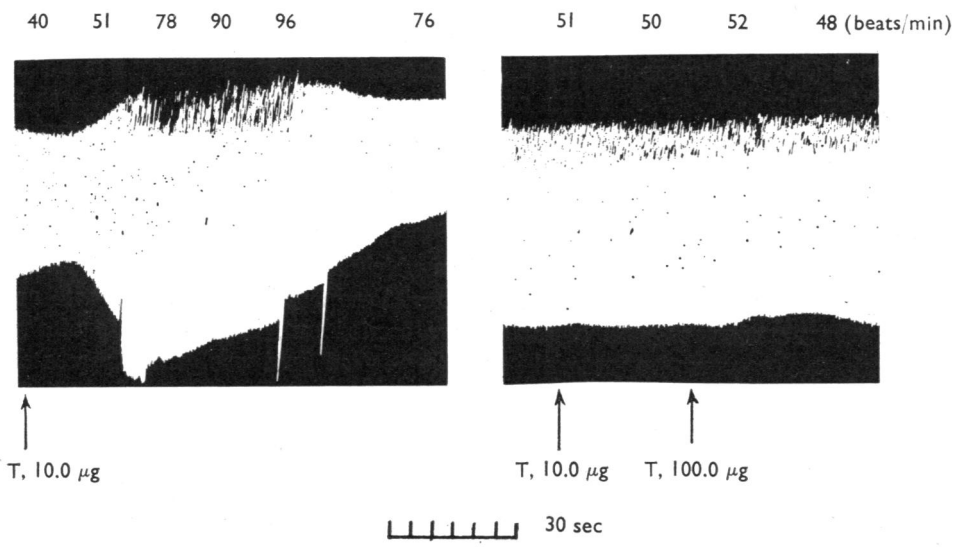

Fig. 3. Contractions of dog atrium in response to tyramine (T). Left, 15 min after the beginning of exposure of the atrium to phenoxybenzamine (2×10^{-5} for 50 min); right, 1 hr after washing out the phenoxybenzamine. Numerals above the records give atrial frequencies (beats/min). Time intervals are 30 sec.

"Repletion" experiments and tyramine action. An effort was made to restore the action of tyramine in an atrium which gave no response to added tyramine after exposure to phenoxybenzamine. Such an atrium was incubated with noradrenaline (4×10^{-6}) for 30 min, and the fluid in the organ-bath then changed several times and the return to the resting frequency and amplitude of atrial contraction was awaited. Tyramine (4×10^{-6}) was still without effect, even though there was a large response to added noradrenaline (4×10^{-8}).

Effect of time on the tyramine response. Tyramine (4×10^{-7}) was added to the atrium every 40 min during an 8 hr period, with very little change in the responses.

Sensitivity of the atrium to 5-hydroxytryptamine. Tyramine might act directly on tryptamine receptors (Gaddum, 1953), or indirectly by releasing 5-hydroxytryptamine. These possible modes of action could be antagonized by phenoxybenzamine (Vane, 1960). The sensitivity of the atrium to 5-hydroxytryptamine was therefore recorded. 5-Hydroxytryptamine (4×10^{-5}) had both positive inotropic and chronotropic actions, the responses being slightly greater than those to noradrenaline

(4×10^{-9}). This indicated that the atrium was relatively insensitive to 5-hydroxytryptamine.

Release of a vasoactive material from the dog isolated atrium by tyramine. In the following experiments, noradrenaline was never added to the atrial preparations, which ensured that any catechol amines liberated came from the natural storage sites of the atrium. The atrium was exposed to dichloroisoprenaline (2×10^{-5}) for 30 min. The fluid in the organ-bath was then changed several times and tyramine (4×10^{-6}) was added for 15 min. The fluid of the bath was then applied to an aortic spiral strip from a reserpinized rabbit.

Tyramine (4×10^{-6}), alone or with the bathing fluid from the atrial preparation not stimulated by tyramine, had little or no effect on the aortic strip. There was a small but definite response at the beginning of one experiment to the control transfer of tyramine with the bathing fluid of the unstimulated atrium. This, however, was exceptional and was much smaller than the positive responses produced when tyramine had been added to the atrial preparation (Fig. 4).

The transfer of the bath fluid from an atrium which had been exposed to tyramine (4×10^{-6}) for 15 min always shortened the arterial strip. Six experiments with an average of four transfers, with controls, were carried out. The response of the aortic strip to the vasoactive material in the transferred fluid was generally greater than that to added noradrenaline (4×10^{-10}).

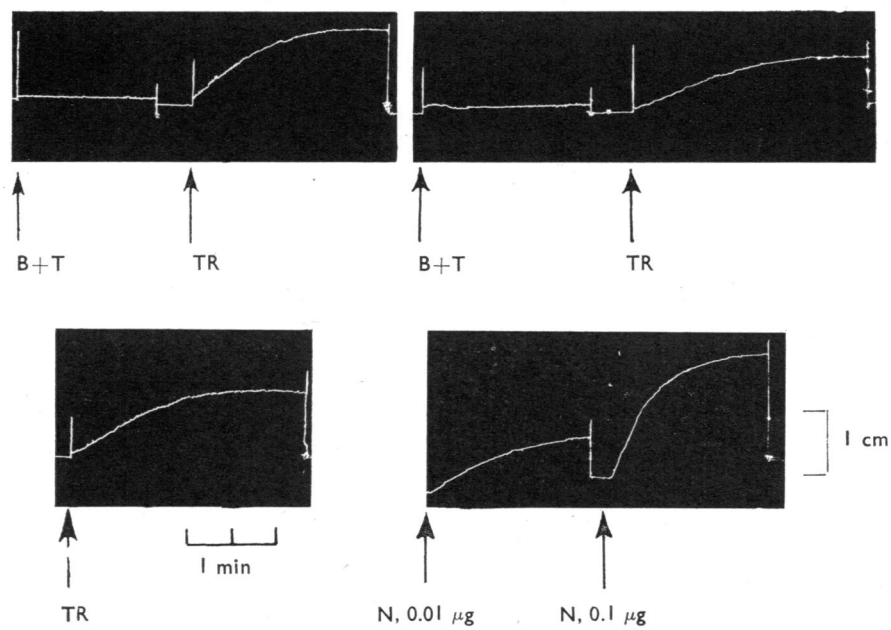

Fig. 4. Contractions of an aortic strip from a reserpinized rabbit. Responses to transfer of fluid (TR), to controls (B+T) and to noradrenaline (N). For details see text. Time intervals are 1 min. The calibration for the height of contraction refers to measurements on the kymograph record.

The timing of the contraction and the relaxation (after washing out the bath) of the aortic strip in response to the vasoactive material was similar to that for added noradrenaline. A striking feature was the constancy of the relationship between the magnitude of the responses and the sensitivity to catechol amines of the aortic strips.

Effect of cyproheptadine on the response of the aortic strip to the vasoactive material. The necessary depletion of the aortic spiral strip of catechol amines by reserpine (to abolish any action of tyramine in the transferred fluid) made the preparation very sensitive to catechol amines. However, it could respond to other substances, for example, histamine, 5-hydroxytryptamine and vasoactive polypeptides. In order to exclude an action of histamine or 5-hydroxytryptamine, cyproheptadine was used. This drug has antihistamine and anti-5-hydroxytryptamine actions (Bodi, Siegler, Gerschenfeld, Brown & Nodine, 1960 ; Stone, Wenger, Ludden, Stavorski & Ross, 1961).

The responses of the aortic strip to noradrenaline, the vasoactive material, histamine and 5-hydroxytryptamine were recorded. The arterial strip was then treated with cyproheptadine (1 μg/l.) for 15 min, and 45 min later the response to added histamine had been abolished and the response to 5-hydroxytryptamine had been reduced to 25% of its former size. The responses to noradrenaline and to the vasoactive material were undiminished.

Transfer of the atrial bath fluid to the guinea-pig ileum and rat fundal strip preparations. Furchgott (1954) found that the aortic strip is generally 100 times less sensitive to histamine and ten times less sensitive to 5-hydroxytryptamine than to catechol amines. Therefore, if histamine or 5-hydroxytryptamine had been the vasoactive material in the transfers, 4×10^{-8} of histamine or 4×10^{-9} of 5-hydroxytryptamine would probably have been the minimum concentrations in the transferred fluid. However, there was no response to the vasoactive material in the fluid transferred either to the guinea-pig ileum or to the rat fundal strip. The guinea-pig ileum is very sensitive to histamine and to 5-hydroxytryptamine (Gaddum, 1953), and gave responses, 4 cm in height on the kymograph, to histamine (4×10^{-9}) and to 5-hydroxytryptamine (4×10^{-9}). The muscle of the rat fundal strip is equally sensitive to 5-hydroxytryptamine (Vane, 1957); a response, 3 cm in height, to 5-hydroxytryptamine (4×10^{-9}) was recorded on the kymograph.

DISCUSSION

Evidence for the release by tyramine of a vasoactive material from the dog atrium (whose receptors are blocked by dichloroisoprenaline) was found consistently. Careful controls eliminated the possibility that the response of the aortic strip was directly due to tyramine in the transferred fluid or to the spontaneous release of a vasoactive material from the atrial preparation. There was, at the beginning of one experiment, a small but definite response to a control transfer in an aortic strip which was very sensitive to catechol amines. Briscoe & Burn (1954), by recirculating a limited volume of Locke solution through the coronary vascular bed of the rabbit isolated heart for 40 min, demonstrated the spontaneous release of catechol amines into the perfusing fluid. The small response to the control

transfer described above is probably due, therefore, to catechol amines spontaneously released from the atrium.

The timing of the contraction and the relaxation (after wash-out of the bath) of the aortic strip exposed to the vasoactive material was similar to that for noradrenaline (Furchgott & Bhadrakom, 1953). The responses of the aortic strip to catechol amines and to the vasoactive material bore a similar relation in all the experiments, which suggests that the vasoactive material was a catechol amine.

Observations on the rat fundal strip and on the guinea-pig ileum indicated that tyramine does not liberate 5-hydroxytryptamine or histamine from the dog atrium, at least in amounts adequate to contract the aortic strip. The negative results of the vasoactive material on the guinea-pig ileum indicate that a vasoactive polypeptide, for example, bradykinin, was unlikely to be responsible for the positive transfers (Rocha e Silva, Beraldo & Rosenfeld, 1949).

The response of the aortic strip to a transfer was generally slightly greater than that to noradrenaline (4×10^{-10}). Since tyramine (4×10^{-6}) produces a response in the dog atrium greater than noradrenaline (4×10^{-8}), the sensitivity of the response to vasoactive material in the transfers means that either a large amount of the catechol amine liberated by tyramine is destroyed, or that a much smaller amount of endogenous material than of added noradrenaline is required to produce the same response.

That the response to tyramine continued after exposure of the atrium to phenoxybenzamine but later disappeared could be due to the gradual release of catechol amines by phenoxybenzamine from storage sites by irreversible competition. The phenoxybenzamine probably slowly displaces the less basic catechol amines from the catechol amine-adenosine triphosphate complex in storage granules and, after a prolonged exposure, the sites of attachment are all irreversibly occupied by the phenoxybenzamine. In such conditions, tyramine, if it liberates catechol amines by a similar mechanism, would produce no response. This view is supported by the observations of Schumann & Philippu (1961) that the addition of tyramine to a suspension of chromaffin granules enhances the spontaneous release of catechol amines but not of adenosine triphosphate, and that the tyramine-induced release of catechol amines is compensated almost stoichiometrically by an uptake of tyramine.

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