Br. J. Pharmac. (1982), **77**, 455–460

EFFECT OF MUSCARINE ON RELEASE OF CATECHOLAMINES FROM THE PERFUSED ADRENAL GLAND OF THE CAT

S.M. KIRPEKAR, J.C. PRAT & M.T. SCHIAVONE

Department of Pharmacology, Downstate Medical Center, State University of New York, 450 Clarkson Avenue, Brooklyn, New York 11203, U.S.A.

1 The secretory effect of muscarine was studied in the perfused adrenal gland of the cat. During perfusion of the adrenal gland with Krebs-bicarbonate solution containing muscarine 480 μM, the rate of catecholamine (CA) secretion was 2.02 ± 0.43 μg/2 min in the first 2 min; thereafter, CA output declined only moderately, to reach about 70% of the initial value after 10 min. Secretory responses to brief infusions of muscarine remained reproducible for at least the first 3 infusions.

2 When the adrenal gland was perfused with muscarine (480 μM), infusions of high K^+, nicotine, or veratridine produced their usual responses. A 100 fold lower dose of muscarine also failed to modify these responses.

3 During perfusion with high K^+, muscarine evoked a secretory response that was only slightly smaller than the response to muscarine alone.

4 It is concluded that muscarine and nicotine activate CA secretion in the cat adrenal gland by independent mechanisms and that the muscarinic response, unlike the nicotinic response, is not readily desensitized.

Introduction

Although the predominant action of acetylcholine as a secretagogue in the adrenal medulla is nicotinic (Dale, 1914), the chromaffin cells additionally contain cholinoceptors of the muscarinic type. Feldberg, Minz & Tsudzimura (1934) showed that muscarine evoked medullary secretion by activating a receptor which is not desensitized by nicotine. More recently, it has been shown that nicotine releases nearly equal amounts of adrenaline and noradrenaline, whereas muscarine and pilocarpine preferentially release adrenaline from the rat adrenal medulla (Douglas & Poisner, 1965). The secretory response to muscarine is Ca^{2+}-dependent (Poisner & Douglas, 1966). However, in the bovine adrenal medulla muscarine does not promote catecholamine (CA) secretion, and diminishes secretory responses evoked by nicotine, possibly by promoting the formation of cyclic guanosine 3′,5′-monophosphate (cyclic GMP, Derome, Tseng, Mercier, Lemaire & Lemaire, 1981).

Nicotinic and muscarinic receptors are both functionally identifiable at the sympathetic nerve terminal, but activation of the muscarinic receptor inhibits adrenergic transmission. Thus, Muscholl and his colleagues have shown that activation of muscarinic receptors reduces the release of noradrenaline from sympathetic nerves by either electrical stimulation or high potassium (Löffelholz & Muscholl, 1969, Dubey, Muscholl & Pfeiffer, 1975).

In the present study, the effect of muscarine on secretion from the cat adrenal medulla was re-examined in the light of its known inhibitory actions in the bovine adrenal medulla, and at the adrenergic nerve terminal. We have shown that in the cat adrenal medulla muscarine does not decrease the secretory responses evoked by high K^+, nicotine, or veratridine.

Methods

Perfusion of isolated adrenal gland

Left and right adrenal glands were isolated and prepared for retrograde perfusion at room temperature, as described by Garcia, Hernandez, Horga & Sanchez-Garcia (1980). The perfusion rate was approx. 1 ml/min. Glands were perfused for approximately 30 min before the start of each experiment.

Perfusion media

Krebs-bicarbonate buffer consisted of (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4 \cdot 7\,H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, and glucose 10. When excess K^+ (as K_2SO_4) was added, an osmotic equivalent of NaCl was concomitantly removed. The perfusion media were equilibrated with a mixture of 95% O_2 and 5% CO_2. The pH was adjusted to 7.4.

0007-1188/82/110455-06 $01.00

Catecholamine assays

The catecholamine content of the perfusate was determined fluorometrically according to the method of Anton & Sayre (1962) without the intermediate alumina-adsorption procedure. Catecholamine values are given as noradrenaline (NA) equivalents.

Analysis of data

Each type of experiment was repeated 3 to 5 times. In Figure 1, the standard error of the mean secretory response is indicated by standard-error bars; standard errors of less than 5% are not indicated. In Figure 2, because the adrenal secretory response to high K^+ tends to decline with repeated applications of the stimulus, the response to high K^+ in the presence of muscarine is compared to a control value estimated as the mean of 2 bracketing responses to high K^+ alone; the bracketing responses were measured approximately 30 min before and 30 min after the response to high K^+ in the presence of muscarine. Control responses for nicotine alone and muscarine alone in Figures 3 and 4, respectively, were similarly estimated.

Material

Acetylcholine and nicotine were obtained from Aldrich Chemical Company (Milwaukee, WI). Muscarine was obtained from Sigma Chemical Company (St. Louis, MO).

Results

The secretory response to muscarine

Muscarine is a relatively weak agonist of CA release in the cat adrenal gland. The output of 2.02 ± 0.43 μg/2 min evoked by muscarine 480 μM in the first 2 min (Figure 1) was 15 to 30% of the initial response evoked by nicotine 60 μM or K^+ 140 mM (Schiavone & Kirpekar, 1982). However, the secretory response evoked by muscarine was much better maintained, and after 10 min the response was dimished by only 25 to 30%; in contrast, responses to high K^+ or nicotine declined by 80% or more after 10 min (Schiavone & Kirpekar, unpublished results). The response upon repeated 2-min infusions of muscarine was also well maintained.

Effect of muscarine on responses induced by high K+, nicotine and veratridine

Acetylcholine inhibits release of NA from adrenergic nerve terminals, and this inhibition has been attributed to an activation of inhibitory muscarinic receptors on the sympathetic nerve terminals (Löffelholz & Muscholl, 1969). It was of interest, then, to examine the effect of muscarine in the cat adrenal gland on secretory responses to various agonists. Figure 2 shows that muscarine 480 μM did not diminish the secretory response to K^+ 140 mM. In 4 experiments, when 140 mM K^+ was added during the 10th to 12th min of muscarine treatment, CA output was greater than the mean of the bracketing control responses to high K^+ alone; after subtracting the basal secretion due to muscarine alone, the response to high K^+ in the presence of muscarine was $132 \pm 12\%$ of the estimated control response to high K^+.

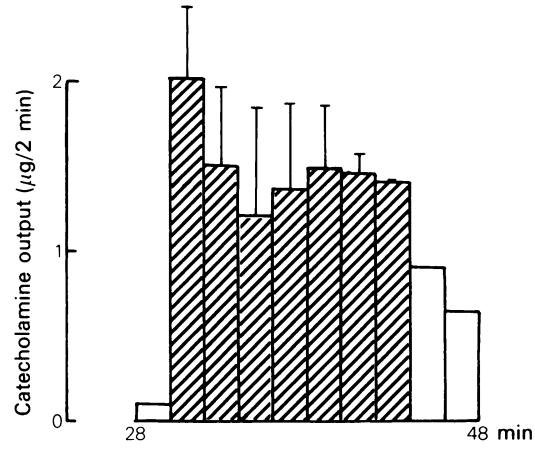

Figure 1 The time course of catecholamine release from cat adrenal glands during continuous perfusion with muscarine 480 μM. The open columns represent 2-min control samples in normal Krebs solution, and the hatched columns depict outputs for 2 min samples when muscarine was present in the Krebs solution. The results are the mean of 5 separate experiments; vertical lines show s.e.mean.

Inasmuch as muscarine (480 μM) released substantial amounts of CA, it seemed possible that this excitatory effect could mask the inhibitory actions of muscarine on secretion evoked by high K^+. The fact that 4.8 μM muscarine also failed to diminish the secretory response to high K^+ argues against this possibility; however, even this low concentration of muscarine demonstrated some secretory effect.

Figure 3 shows that muscarine also does not appreciably modify nicotine-evoked secretion. The second response to nicotine 60 μM, in the presence of muscarine (480 μM), was about 90% of the mean of the bracketing control responses. In 4 experiments, the net second response evoked by nicotine in the presence of muscarine was $43.6 \pm 6.4\%$ of the first response; in another series of 4 experiments with nicotine alone, the second response was $51.1 \pm 12.2\%$ of the first response. Thus it appears

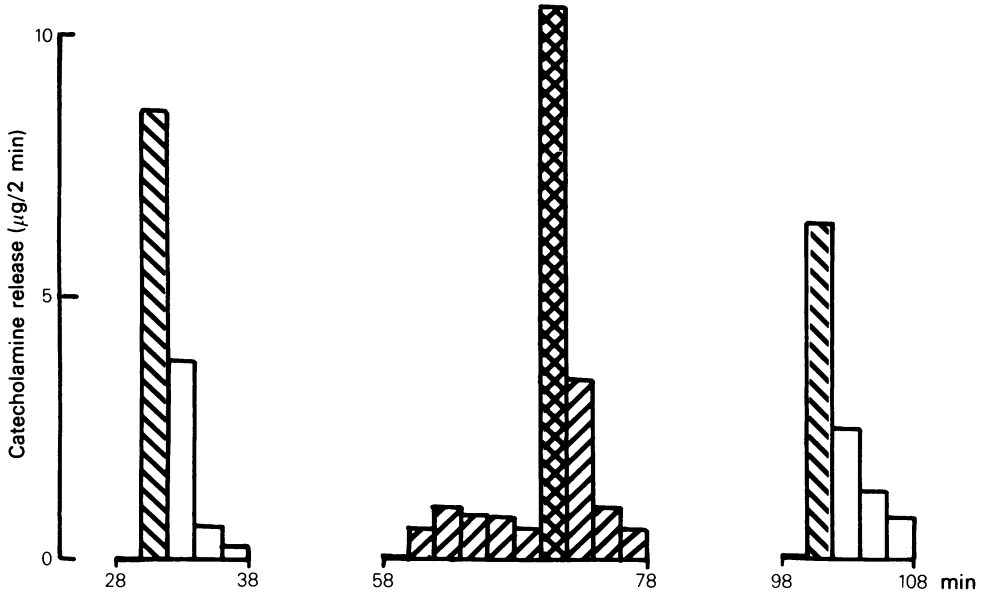

Figure 2 The stimulant effect of high K^+ during perfusion of cat adrenal glands with muscarine. In the first and third trials, the gland was perfused for 2 min with a modified Krebs solution containing K^+ 140 mM (▨). In the second trial, the gland was exposed to muscarine 480 μM (▨) for 18 min, and K^+ 140 mM was added during the 10th to 12th min of muscarine treatment. A typical result from 1 of 4 experiments is shown.

that muscarine does not significantly dimish the response to nicotine.

The effect of muscarine on the secretory response to veratridine was also examined. When the gland was exposed to veratridine 30 μM for 2 min, the rate of CA secretion was not only increased during the 2-min perfusion of the drug, but remained high for at least an additional 8 min during subsequent perfusion with normal Krebs solution (Kirpekar & Prat, 1979). Muscarine affected neither the initial response nor the delayed response to veratridine.

Effect of high K^+ on the secretory response to muscarine

When acetylcholine is introduced several min after the start of high K^+ treatment, CA output evoked by acetylcholine is diminished to approximately 10% of the normal response. Sensitivity to nicotine, however, is completely obliterated in the presence of high K^+ (Schiavone & Kirpekar, unpublished results). To examine the interaction between depolarization and the muscarinic component of acetylcholine stimulation, the effect of muscarine on the late phase of K^+-evoked secretion was examined. Figure 4 shows a typical experiment in which muscarine (480 μM) was introduced at the tenth min of infusion with K^+ 140 mM. At this time, the response to high K^+ already had been markedly reduced, but addition of muscarine enhanced the late response. In 3 experiments, the response to muscarine in the presence of high K^+ was 75.2 ± 7.5% of the estimated control response to muscarine alone in the same glands. Thus, the secretory response to muscarine in high K^+ solution appears to be much better maintained than that of nicotine or acetylcholine.

Discussion

The stimulant action of muscarine on adrenomedullary secretion and its blockade by atropine has been known for some time (Feldberg *et al.*, 1934). Douglas & Poisner extended these observations, showing that pilocarpine and muscarine preferentially release adrenaline from the perfused adrenal medulla of the cat (Douglas & Poisner, 1965), and that the secretory response to muscarine, pilocarpine, or methacholine is Ca^{2+}-dependent (Poisner & Douglas, 1966).

In the present results we have shown that the enhancement of secretion by muscarine is maintained at a fairly constant level for at least 10 min, in contrast to nicotine, the response to which, at high doses is initially 2 to 4 times greater but then falls by more than 80% after 10 min. Secretion during the 8th to 10th min of muscarine infusion is nearly 70% of the initial response. Moreover, on repeated brief infusions the secretory response to muscarine is not appreciably diminished. Thus, although muscarine appears to be a weaker secretagogue than nicotine,

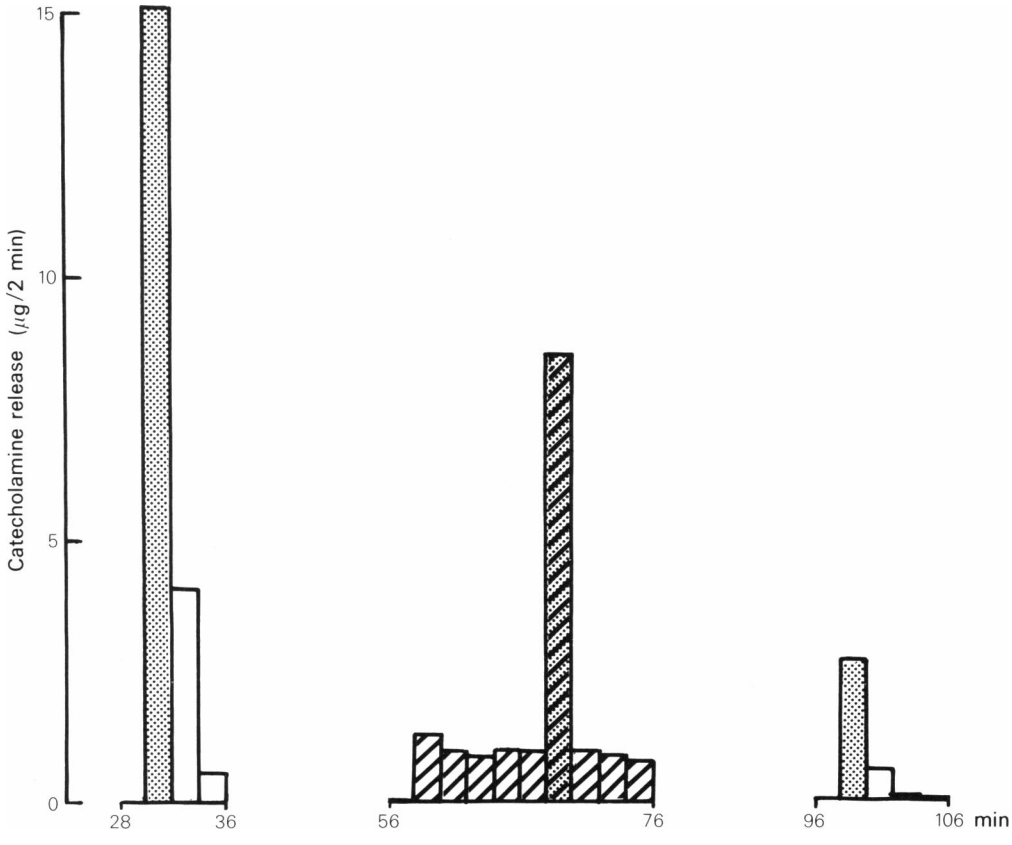

Figure 3 The stimulant effect of nicotine during perfusion of cat adrenal glands with muscarine. In the first and third trials, the gland was perfused for 2 min with a normal Krebs solution containing nicotine 60 μM (stippled columns). In the second trial, the gland was exposed to muscarine 480 μM for 18 min (hatched columns) and nicotine was added during the tenth to twelfth min of muscarine treatment. A typical result from 1 of 4 experiments is shown.

the muscarine response, unlike the nicotinic response, does not appear to be limited by receptor desensitization.

We have also shown that muscarine does not inhibit the secretory response induced by high K^+, nicotine, or veratridine. Each agent enhances Ca^{2+} permeability as a consequence of depolarization: high K^+ depolarizes the cell by lowering the ratio $[K]_i/[K]_o$, and nicotine activates a receptor which opens a nondiscriminating cation channel (Douglas, 1975), while veratridine depolarizes the cell by activating tetrodotoxin-sensitive Na^+ channels (Kirpekar & Prat, 1979). Thus, the present observations suggest that muscarine does not reduce Ca^{2+} availability in the adrenal chromaffin cell, as it appears to act at postganglionic sympathetic nerve terminals (Dubey *et al.*, 1975). In the present study, however, the inhibitory action of muscarine may have been obscured by the measurement of 'total catecholamines' rather than separate determinations of adrenaline and noradrenaline. Inasmuch as muscarinic agonists selectively enhance secretion from adrenaline-containing chromaffin cells (Douglas & Poisner, 1965), the possibility that muscarine selectivity depresses secretory responses to high K^+, nicotine, or veratridine in noradrenaline-containing cells must also be examined before we can rule out an inhibitory effect for muscarine in the cat adrenal medulla.

Muscarine may activate voltage-dependent Ca^{2+} permeability to promote secretion. In the isolated chromaffin cells of the gerbil, the depolarizing effect of pilocarpine is blocked by atropine alone; depolarization by acetylcholine is only partially blocked when hexamethonium is added alone, but completely blocked when atropine and hexamethonium are added together (Douglas, Kanno & Sampson, 1967). Atropine also blocks the depolarizing effect of acetylcholine in rat adrenal chromaffin cells (Brandt, Hagiwara, Kidokoro & Miyazaki, 1976). It is not

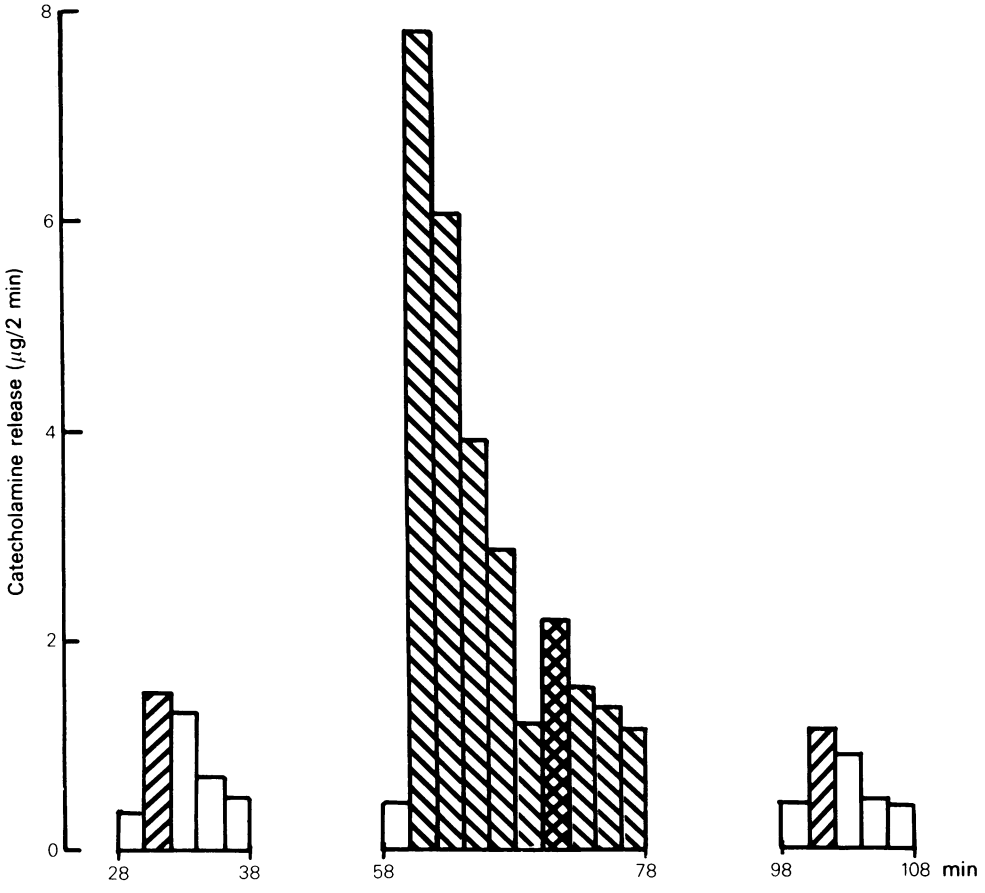

Figure 4 The stimulant effect of muscarine during perfusion of cat adrenal glands with high K^+. In the first and third trials, the gland was perfused for 2 min with a normal solution containing muscarine 480 μM (▨). In the second trial, the gland was exposed to K^+ 140 mM for 18 min (▧), and muscarine was added during the tenth to twelfth min of high K^+ treatment. A typical result from 1 of 5 experiments is shown.

known whether muscarinic stimulation likewise depolarizes chromaffin cells of the cat adrenal medulla. However, most of the secretory response to muscarine in the cat adrenal medulla is probably not associated with voltage-dependent Ca^{2+} permeability, since the secretory effect of muscarine is only reduced by 20 to 30% in the presence of K^+ 140 mM. This suggests that muscarinic stimulation in the cat adrenal medulla activates a Ca^{2+} permeability which is distinct from voltage-sensitive Ca^{2+} permeability.

In isolated chromaffin cells of the bovine adrenal gland, nicotinic and muscarinic cholinoceptors have also been functionally distinguished; however, in these cells, as at the sympathetic nerve terminals, muscarinic agonists inhibit the secretory response to nicotine (Derome *et al.*, 1981). It also has been shown that nicotine or high K^+ increase CA secretion without altering the level of cyclic GMP, whereas muscarinic agonists cause a 4 to 5 fold increase in cyclic GMP (Schneider, Cline & Lemaire, 1979; Yanagihara, Isosaki, Ohuchi & Oka, 1979; Derome *et al.*, 1981). These studies demonstrate that stimulation of muscarinic receptors can cause different types of responses in chromaffin cells obtained from different species of animals. It would be interesting to determine whether the secretory response to muscarine in the cat adrenal medulla is accompanied by changes in the levels of cyclic AMP or cyclic GMP.

This work was supported by USPHS grant HL22170 and Training Grant 5T32GM07615.

References

ANTON, A.H. & SAYRE, D.F. (1962). A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138,** 360–375.

BRANDT, B.L., HAGIWARA, S., KIDOKORO, Y., & MIYAZAKI, S. (1976). Action potentials in the rat chromaffin cell and effects of acetylcholine. *J. Physiol.*, **263,** 417–439.

DALE, H.H. (1914). The action of certain esters and ethers of choline and their relation to muscarine. *J. Pharmac. exp. Ther.*, **6,** 147–190.

DEROME, G., TSENG, R., MERCIER, P., LEMAIRE, I., & LEMAIRE, S. (1981). Possible muscarinic regulation of catecholamine secretion mediated by cyclic GMP in isolated bovine adrenal chromaffin cells. *Biochem Pharmac.*, **30,** 855–860.

DOUGLAS, W.W. (1975). Secretomotor control of adrenal medullary secretion: synaptic, membrane, and ionic events in stimulus-secretion coupling. In *Handbook of Physiology*, Section 7: *Endocrinology*, ed. Greep, R.O. & Astwood, E.B. Vol. VI (*Adrenal Gland*). pp. 367–388. Washington, D.C.: American Physiological Society.

DOUGLAS, W.W., KANNO, T., & SAMPSON, S.R. (1967). Effects of acetylcholine and other medullary secretagogues and antagonists on the membrane potential of adrenal chromaffin cells: an analysis employing techniques of tissue culture. *J. Physiol.*, **188,** 107–120.

DOUGLAS, W.W. & POISNER, A.M. (1965). Preferential release of adrenaline from the adrenal medulla by muscarine and pilocarpine. *Nature*, **208,** 1102–1103.

DUBEY, M.P., MUSCHOLL, E., & PFEIFFER, A. (1975). Muscarinic inhibition of potassium-induced noradrenaline release and its dependence on the calcium concentration. *Naunyn-Schmiedebergs Arch. Pharmac.*, **291,** 1–15.

FELDBERG, W., MINZ, B. & TSUDZIMURA, H. (1934). The mechanism of the nervous discharge of adrenaline. *J. Physiol.*, **81,** 286–304.

GARCIA, A.G., HERNANDEZ, M., HORGA, J.F. & SANCHEZ-GARCIA, P. (1980). On the release of catecholamines and dopamine-β-hydroxylase evoked by ouabain in the perfused cat adrenal gland. *Br. J. Pharmac.*, **68,** 571–583.

KIRPEKAR, S.M. & PRAT, J.C. (1979). Release of catecholamines from perfused cat adrenal gland by veratridine. *Proc. natn. Acad. Sci. U.S.A.*, **76,** 2081–2083.

LÖFFELHOLZ, K. & MUSCHOLL, E. (1969). A muscarinic inhibition of the noradrenaline release evoked by postganglionic sympathetic nerve stimulation. *Naunyn-Schmiedebergs Arch. Pharmac.*, **265,** 1–15.

POISNER, A.M. & DOUGLAS, W.W. (1966). The need for calcium in adrenomedullary secretion evoked by biogenic amines, polypeptides, and muscarinic agents. *Proc. Soc. exp. Biol. Med.*, **123,** 62–64.

SCHNEIDER, A.S., CLINE, H.T. & LEMAIRE, S. (1979). Rapid rise in cyclic GMP accompanies catecholamine secretion in suspensions of isolated adrenal chromaffin cells. *Life Sci.*, **24,** 1389–1394.

YANAGIHARA, N., ISOSAKI, M., OHUCHI, T. & OKA, M. (1979). Muscarinic receptor-mediated increase in cyclic GMP level in isolated bovine adrenal medullary cells. *FEBS Letters*, **105,** 296–298.

(Received March 3, 1982.
Revised June 22, 1982)