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REFERENCES

1. L. Shuster, F. Quimby, A. Bates and M. L. Thompson, *Life Sci.* **20**, 1035 (1977).
2. M. A. Evans, C. Dwivedi and R. D. Harbison, in *Cocaine and Other Stimulants* (Eds. E. H. Ellinwood, Jr. and M. M. Kilbey), p. 253. Plenum Press, New York (1977).
3. M. W. Kloss, G. M. Rosen and E. J. Rauckman, *Toxic. appl. Pharmac.* **65**, 75 (1982).
4. E. J. Rauckman, M. W. Koss and G. M. Rosen, *Can. J. Chem.* **60**, 1614 (1982).
5. M. W. Kloss, G. M. Rosen and E. J. Rauckman, *Toxic. Lett.* **15**, 65 (1983).
6. G. M. Rosen, M. W. Kloss and E. J. Rauckman, *Molec. Pharmac.* **22**, 529 (1982).
7. R. W. Freeman and R. D. Harbison, *Biochem. Pharmac.* **30**, 777 (1981).
8. F. Wroblewski and J. S. LaDue, *Proc. Soc. exp. biol. Med.* **91**, 569 (1956).
9. J. B. Schenkman and D. L. Cinti, in *Methods in Enzymology* (Eds. S. Fleischer and L. Packer), Vol. 52, p. 83. Academic Press, New York (1978).
10. T. Omura and R. Sato, *J. biol. chem.* **239**, 2370 (1964).
11. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
12. M. Danhof, E. de Groot-van der Vis and D. D. Breimer, *Pharmacology* **18**, 210 (1979).
13. P. Armitage, *Statistical Methods in Medical Research*, p. 269. John Wiley, New York (1971).
14. B. B. Brodie, J. Axelrod, R. Soberman and B. B. Levy, *J. biol. Chem.* **179**, 25 (1949).
15. R. Soberman, B. B. Brodie, B. B. Levy, J. Axelrod, V. Hollander and J. M. Steele, *J. biol. Chem.* **179**, 131 (1949).
16. B. B. Brodie and J. Axelrod, *J. Pharmac. exp. Ther.* **98**, 97 (1950).
17. R. A. Branch, D. G. Shand, G. R. Wilkinson and A. S. Nies, *J. clin. Invest.* **53**, 1101 (1974).
18. E. S. Vesell and J. C. Page, *Science* **161**, 72 (1968).
19. E. S. Vesell and J. C. Page, *J. clin. Invest.* **47**, 2657 (1968).
20. I. H. Stevenson, *Br. J. clin. Pharmac.* **4**, 261 (1977).
21. E. S. Vesell, *Clin. Pharmac. Ther.* **26**, 275 (1979).

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Catecholamine secretion by perfused bovine adrenal medulla in response to nicotinic activation is inhibited by muscarinic receptors

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Increased labelling of phosphatidylinositol and phosphatidic acid with ^{32}P is seen as a response to activation of various cell surface receptors. In the bovine adrenal medulla these phospholipid changes are associated with muscarinic but not nicotinic cholinergic receptors [1, 2]. It is generally agreed that in the bovine medulla catecholamine secretion is mediated by nicotinic receptors but there are conflicting views on the function of the muscarinic receptors. Wilson and Kirshner [3] claimed that the acetylcholine receptors of bovine adrenal medulla are entirely nicotinic. In their hands, the muscarinic drug pilocarpine at concentrations between 10^{-5} and 3×10^{-3} M did not stimulate catecholamine secretion by the perfused gland. Gothert *et al.* [4], on the other hand, obtained a secretory effect dependent upon Ca^{2+} with 1.4×10^{-3} M pilocarpine in similar experiments.

Binding studies show the presence of muscarinic receptors in the bovine gland [5] and studies with isolated chromaffin cells suggest that activation of these receptors inhibits the nicotinic secretion of catecholamine, possibly by increasing c-GMP concentration [6]. Using cultured bovine chromaffin cells, Fisher *et al.* [2] showed that muscarinic receptors do not enhance catecholamine secretion.

It seemed likely then that in the bovine adrenal medulla nicotinic activation promotes catecholamine release while muscarinic activation inhibits this release. The experiments reported below were designed to test this hypothesis using the perfused gland.

Bovine adrenals were removed within 15 min of death and transported to the laboratory on ice. Connective tissue and fat were removed and two incisions made in the cortical tissue without damaging the medulla, so that the perfusion fluid could flow out. Glands were cannulated and perfused in a retrograde manner via the central vein opening at a flow rate of 4 ml/min. The Locke's solution used had the following composition: 154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 6.0 mM $NaHCO_3$ and 10.0 mM glucose. The perfusion fluid temperature was kept at 35° and flow was maintained by a Watson–Marlow MC/10 peristaltic metering pump. The fluid was continuously gassed with O_2–CO_2 (95:5). The perfusion system consisted of two glands, each with its own reservoir.

After perfusion for 20 min in this way, the perfusate was collected in 2 min samples (i.e. from time 0 in Figs. 1 and 2) and these were stored at 4° for analysis. Catecholamines in the perfusate were estimated as described previously [7]. To stimulate secretion, a total of 4 ml 3×10^{-4} M nicotine was injected 1.0 ml at a time into the tube carrying perfusion fluid just before it entered the gland. There was an interval of 30 sec between injections. A further identical nicotine injection was given later as shown in the figures. Both glands received the injections, the first gland being

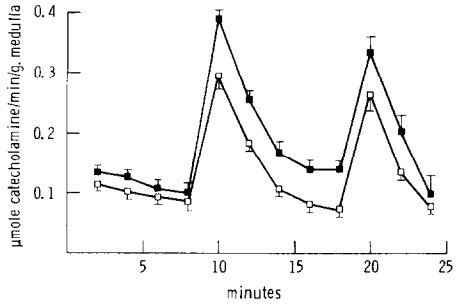

Fig. 1. Catecholamine secretion in response to nicotine. The nicotine (4 ml of 3×10^{-4} M) was injected at 8 and 18 min. ■, Perfusion with normal Locke's solution; □, perfusion with Locke's solution containing 10^{-4} M pilocarpine. Each point is the mean of results from three perfusion experiments. Vertical bars indicate S.D.

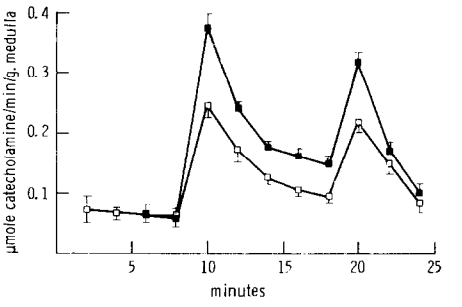

Fig. 2. Catecholamine secretion in response to nicotine injected (4 ml of 3×10^{4} M) at 8 and 18 min. ■, Perfusion with normal Locke's solution; □, perfusion with Locke's solution containing 10^{-4} M methacholine. Each point is the mean of results from three perfusion experiments. Vertical bars indicate S.D.

Table 1. Effect of muscarinic drugs on catecholamine secretion by the bovine adrenal medulla

Treatment	Catecholamine secretion* (μmole/g medulla)	Inhibition (%)
Nicotine; perfusion with Locke's solution	1.11 ± 0.07	—
Nicotine; perfusion with Locke's solution containing 10^{-4} M pilocarpine	0.64 ± 0.02	42.4
Nicotine; Locke's solution	1.43 ± 0.08	—
Nicotine; Locke's solution containing 10^{-4} M methacholine	0.80 ± 0.04	43.8

* The catecholamine secretion figures represent the total increased secretion in the 8 min period after one nicotine administration (mean of results from three glands in each case ± S.D.). The nicotine dosage was 4 ml of 3×10^{-4} M solution. By Student's *t*-test, the inhibitions were statistically significant at $P < 0.001$.

perfused with the usual Locke's solution, the second with Locke's solution containing 10^{-4} M pilocarpine (Fig. 1) or methacholine (Fig. 2).

As shown in Table 1, both these muscarinic drugs significantly reduced nicotinic stimulation of catecholamine secretion. This confirms the presence of muscarinic receptors in the bovine adrenal medulla. Their inhibitory effect is in keeping with the results of Derome *et al.* [6] and Fisher *et al.* [2].

Phosphatidylinositol changes accompany activation of this inhibitory muscarinic receptor just as they accompany activation of stimulatory muscarinic receptors such as those of the parotid [8]. It is difficult to reconcile these observations with the theory of Michell that hydrolysis of phosphatidylinositol [9] or its phosphorylated derivatives [10] controls entry of calcium into cells responding to muscarinic stimuli.

While muscarinic receptors appear to be inhibitory in the bovine adrenal medulla, they may stimulate catecholamine secretion in other species such as the cat [11] and the chicken [12].

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REFERENCES

1. N. A. Mohd. Adnan and J. N. Hawthorne, *J. Neurochem.* **36**, 1858 (1981).
2. S. K. Fisher, R. W. Holz and B. W. Agranoff, *J. Neurochem.* **37** 491 (1981).
3. S. P. Wilson and N. Kirshner, *J. Neurochem.* **28**, 687 (1977).
4. M. Gothert, W. Dorn and I. Loewenstein, *Naunyn-Schmiedeberg's Archs exp. Path. Pharmac.* **294**, 239 (1976).
5. S. O. Kayaalp and N. H. Neff, *Neuropharmacology* **18**, 909 (1979).
6. G. Derome, R. Tseng, P. Mercier, I. Lemaire and S. Lemaire, *Biochem. Pharmac.* **30**, 855 (1981).
7. N. Azila and J. N. Hawthorne, *Biochem. J.* **204**, 291 (1982).
8. L. M. Jones and R. H. Michell, *Biochem. J.* **142**, 583 (1974).
9. R. H. Michell, *Biochim. biophys. Acta* **415**, 81 (1975).
10. R. H. Michell, *Nature, Lond.* **296**, 492 (1982).
11. W. W. Douglas and A. M. Poisner, *Nature, Lond.* **208**, 1102 (1965).
12. F. H. Ledbetter and N. Kirshner, *Biochem. Pharmac.* **24**, 967 (1975).