Isoprenaline ($5–20 \times 10^{-9}$ g/ml) decreased the slowing of the heart response to vagal stimulation and to exogenous acetylcholine. The duration of action of this latter acetylcholine effect was shortened by isoprenaline. The β-adrenoceptor blocking agent 4-(2-hydroxy-3-isopropylaminopropoxy) indole (LB46) (2×10^{-8} g/ml) abolished the effect of isoprenaline in reducing vagal bradycardia. This seemed to exclude the possibility of the damage or fatigue of the vagus nerve as well as the exhaustion of the acetylcholine stores, provided that LB46 did not exert some unsuspected " facilitation " upon nerve activity or transmitter release. In control experiments in the absence of isoprenaline, there was no tachyphylaxis to acetylcholine, which accords with the view that the inhibition of the acetylcholine effect was due to isoprenaline.

Acetylcholine receptors of invertebrate neurones

G. A. KERKUT, LYNNE C. NEWTON, R. M. PITMAN, R. J. WALKER and G. N. WOODRUFF, *Department of Physiology and Biochemistry, The University of Southampton, Southampton*

In this study we have compared the acetylcholine receptors in the nervous systems of three species. The neurones tested were the giant cells (Retzius, 1891) in the ventral nerve cord of *Hirudo medicinalis*, cells in the visceral ganglion of *Helix aspersa* and cells in the sixth abdominal ganglion of *Periplaneta americana*. Action potentials were recorded intracellularly by methods previously described (Kerkut & Walker, 1967; Kerkut, Pitman & Walker, 1969; Walker & Hedges, 1968). Drugs were applied either by addition to the bath or iontophoretically. The potencies of the agonists are expressed as equipotent molar ratios relative to the carbachol threshold. The drugs used were carbachol, acetylcholine (ACh), 1,1-dimethyl-4-phenylpiperazinium (DMPP), 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethyl ammonium (McN-A-343), muscarone, nicotine and furmethide (furtrethonium).

In the snail there are two types of cell responsive to acetylcholine. Some neurones (called D cells) are depolarized and excited by acetylcholine, whereas other cells (called H cells) are hyperpolarized and inhibited by acetylcholine. Our results (Table I) suggest that each type of cell contains both muscarinic and nicotinic receptors, although McN-A-343 has little effect on D cells.

In the cockroach sixth abdominal ganglion all of the neurones tested were depolarized and excited by acetylcholine. Nicotinic agonists were powerful stimulants of these cells, but muscarinic agonists were less potent, indicating that the receptors are mainly nicotinic (Table 1).

In the Retzius cells of the leech the nicotinic agonists produced a depolarization similar to that produced by carbachol. In contrast the muscarinic agonists muscarone, furmethide and McN-A-343 caused inhibition when added in threshold amounts. In higher concentrations the muscarinic agonists sometimes produced excitation.

In all three species the effects produced by the iontophoretic application of carbachol were similar to those obtained when the agonist was applied by addition to the bath.

TABLE 1. *Mean equipotent molar ratios (EPMR) of compounds with excitatory and inhibitory activity on neurones of the snail, leech and cockroach*

	Mean EPMR for excitation							Mean EPMR for inhibition						
	Carb-achol	ACh	DMPP	Nico-tine	Musc-arone	McN-A-343	Furm-ethide	Carb-achol	ACh	DMPP	Nico-tine	Musc-arone	McN-A-343	Furm-ethide
Snail neurones D cells	1	2	55	1	11	—	23	—	—	—	—	—	—	—
Snail neurones H cells	—	—	—	—	—	—	—	1	1·2	12	0·5	10	16	44
Leech Retzius cells	1	480	3	3	*	*	*	—	—	—	—	90	3	190
Cockroach neurones	1	515	Not tested	0·05	3	90	37	—	—	—	—	—	†	—

All values refer to experiments in which the agonists were applied by addition to the bath and are the means of at least six experiments.

— indicates that the agonist did not have the stated effect on the neurones. In the presence of eserine (10^{-8}M) the equipotent molar ratios for ACh on leech and cockroach neurones were greatly reduced.

* The muscarinic agonists inhibited the leech neurones in threshold amounts; in higher concentrations they sometimes, but not always, caused excitation.

† In two experiments the initial excitation was replaced by inhibition after several additions.

REFERENCES

KERKUT, G. A., PITMAN, R. M. & WALKER, R. J. (1969). Iontophoretic application of acetylcholine and GABA onto insect central neurones. *Comp. Biochem. Physiol.*, **31**, 611–633.

KERKUT, G. A. & WALKER, R. J. (1967). The action of acetylcholine, dopamine and 5-hydroxytryptamine on the spontaneous activity of the cells of Retzius of the leech, *Hirudo medicinalis*. *Br. J. Pharmac. Chemother.*, **30**, 644–654.

RETZIUS, G. (1891). Zur Kenntnis des centralen nerven systems der Würmer. *Biol. Unters.* (*N.F.*), **2**, 1–28

WALKER, R. J. & HEDGES, A. (1968). The effect of cholinergic agonists on the spontaneous activity of neurones of *Helix aspersa*. *Comp. Biochem. Physiol.*, **24**, 355–376.

Electron microscopical examination of the junction between the myenteric plexus and the longitudinal muscle of the guinea-pig ileum

G. GABELLA (introduced by H. W. KOSTERLITZ), *Department of Anatomy, University College, London*

Electron microscopical examination of the longitudinal muscle of the guinea-pig ileum confirms previous observations (Paton & Zar, 1968) that this layer is usually devoid of nerve fibres. The problem arises as to how excitatory and inhibitory stimuli can reach the longitudinal muscle cells.

Anatomical evidence shows five different possibilities:

(*a*) Nerve fibres right at the surface of ganglia in the myenteric plexus show " synaptic " vesicles, frequently forming aggregations under the plasma membrane, which might release the transmitter diffusing towards the longitudinal muscle cells.

(*b*) Secondary and tertiary strands of the myenteric plexus have vesicle-containing fibres at the surface of the bundle at a short distance from the more internally situated longitudinal muscle cells.

(*c*) Junctions have been found between muscle cells of the circular muscle layer and muscle cells of the longitudinal muscle layer; these junctions generally have finger-like processes.

(*d*) There are junctions between smooth muscle cells and the so-called " interstitial cells " (Cajal) which, at other parts of their surface, are connected with nervous structures.

(*e*) Finger-like processes may protrude from the innermost longitudinal muscle cells and closely approach the external surface of the ganglion.

Quantitative determinations of these various neuromuscular structures are in progress and possible functional implications will be discussed.

I thank the Wellcome Trust for a Fellowship.

REFERENCE

PATON, W. D. M. & ZAR, M. A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle fibres. *J. Physiol., Lond.*, **194**, 13–33.

Measurement and printout of average heart rate using digital techniques

A. K. ARMITAGE, C. A. GRANT, P. N. LEE, C. M. SELLERS and A. WILSON, *Department of Pharmacology, Tobacco Research Council Laboratories, Harrogate*

The equipment to be demonstrated was developed in order to study the effects on heart rate of cigarette and cigar smoking. Frequent, accurate and rapid measurements