REFERENCES

BERETTA, C., GLÄSSER, A. H., NOBILI, M. B. & SILVESTRI, R. (1965). *J. Pharm. Pharmac.*, **17**, 423–428.

HOLLIFIELD, G. & PARSON, W. (1962). *J. clin. Invest.*, **41**, 250–253.

JESPERSEN, S. & SCHEEL-KRÜGER, J. (1970). *J. Pharm. Pharmac.*, **22**, 637–638.

LE DOUAREC, J. C., SCHMITT, H. & LAUBIE, M. (1966). *Archs int. Pharmacodyn. Thér.*, **161**, 206–232.

OPITZ, K. (1967). *Arch. Pharmak. exp. Path.*, **259**, 56–65.

Acetylcholine and "auto-inhibition"

The evidence that the release of noradrenaline from sympathetic fibres is due to the acetylcholine released by the nerve impulse, has now been strengthened by the work of Eränkö, Rechardt & others (1970), and by that of Malik (1970). The former have stained the pineal gland of the rat by the thiocholine method, and have shown that adrenergic terminals seen in electron micrographs are closely invested with acetylcholinesterase. The pineal gland is innervated entirely by fibres from the superior cervical ganglion, and when the ganglia of both sides are removed, both the acetylcholinesterase and the small granular vesicles containing noradrenaline disappear.

Malik perfused the superior mesenteric artery and its branches in the rat, and recorded the constrictor response in the arteries to postganglionic stimulation. He found that the response to stimulation of frequencies from 1 to 6 s was increased when anticholinesterases were added to the perfusion fluid, and in about 100 experiments showed that the increase was greatest at the lowest frequency, diminishing as the frequency rose until at 6 s the increase was imperceptible. Since the investigations of Eränkö & others (1970) and of Malik (1970) provide very clear evidence, the recent work of Löffelholz (1971) requires consideration.

Löffelholz has carried out experiments on the isolated heart of the rabbit, in which the sympathetic postganglionic nerves were stimulated, and the noradrenaline appearing in the effluent was measured. In the course of these experiments either acetylcholine (plus atropine) was added to the perfusion fluid for a short period, or nicotine, or DMPP, was added, and the noradrenaline released by these substances was measured.

The concentration of acetylcholine infused was large, $2{\cdot}1 \times 10^{-4}$M, and this caused a release of a large amount of noradrenaline. However this release was very brief, continuing for 5 to 10 s only, although the infusion of acetylcholine was maintained for 9 min.

The author considered that the cessation of noradrenaline release after 5 to 10 s was due to "auto-inhibition", the receptors for acetylcholine being blocked by the infusion. The important point was that he found that during this "auto-inhibition" the response to sympathetic stimulation was unchanged. He said "when the nicotinic block was established, the noradrenaline released by electrical stimulation was not inhibited", and his implication was that the receptors on which acetylcholine acted to release noradrenaline were not involved in the release of noradrenaline by sympathetic stimulation.

Since the evidence from anticholinesterases shows that sympathetic stimulation involves receptors for acetylcholine, it follows that the "auto-inhibition" must occur at some other point. The same problem was raised by the experiments of Daly & Scott (1961) who found that acetylcholine, injected into the splenic artery, released noradrenaline, but that this release was blocked by hexamethonium, whereas the response to stimulation of the splenic nerves was not. It seemed possible that the

block of the injected acetylcholine by hexamethonium was a block of the access of acetylcholine to the receptors, and not a block of the receptors themselves. Burn & Gibbons (1964) decided to test this, and did so by choosing bretylium which is chemically similar to acetylcholine. They used the Finkleman (1930) preparation of the rabbit ileum to discover whether hexamethonium would block the action of bretylium in abolishing the response to sympathetic stimulation. They found that it did, from which it followed that hexamethonium was blocking the access of bretylium to the receptors, and thus there was reason to think that hexamethonium also blocked the access of acetylcholine to the receptors.

In the course of these experiments, observations were made to find out if the tertiary compound pempidine, which is a ganglion-blocking agent having a chemical resemblance to nicotine, had any effect in blocking sympathetic nerve endings. When pempidine was added to the bath containing the Finkleman preparation of the rabbit ileum, the following result was consistently obtained. In a concentration of 5×10^{-5} g/ml, pempidine in the first 10 min acted like bretylium in causing a gradually increasing block of sympathetic stimulation. Then the blocking action stopped although not more than 50% complete, and during the next hour the block became less. In the next 2 h the block became complete. The observations appeared to indicate that at first pempidine reached the nicotinic receptors on which the sympathetic impulse acts and began to block them. Then further access of pempidine to these receptors was prevented by pempidine itself, due to "auto-inhibition".

To return to the experiments of Löffelholz, he found in some of his experiments on the perfused heart that the amount of noradrenaline released by sympathetic stimulation was very much increased during the infusion of acetylcholine. This occurred when the concentration of acetylcholine infused was $5{\cdot}5 \times 10^{-5}$M, less than the concentration used previously, and when it had been infused for 1 min only. He could not offer a satisfactory explanation for this, but I think it likely that the infused acetylcholine added its effect to that released by stimulation, to release, in turn, more noradrenaline. We know that acetylcholine is concerned in the release of noradrenaline in the isolated rabbit heart from the work of Huković (1966).

University of Oxford, J. H. BURN
Oxford, U.K.
April 13, 1971

REFERENCES

BURN, J. H. & GIBBONS, W. R. (1964). *Br. J. Pharmacol. Chemother.*, **22**, 549–557.
DALY, M. DE B. & SCOTT, M. J. (1961). *J. Physiol., Lond.*, **156**, 246–259.
ERÄNKÖ, O., RECHARDT, L., ERÄNKÖ, L. & CUNNINGHAM, A. (1970). *Histochem. J.*, **2**, 479–489.
FINKLEMAN, B. (1930). *J. Physiol., Lond.*, **70**, 145–157.
HUKOVIĆ, S. (1966). *Br. J. Pharmac. Chemother.*, **28**, 273–281.
LÖFFELHOLZ, K. (1970). *Arch. Pharmak.*, **267**, 64–73.
MALIK, K. U. (1970). *Circulation Res.*, **27**, 647–655.

The spectrophotometric determination of ampicillin in body fluids

Smith, De Grey & Patel (1967) described a specific spectrophotometric method for the determination of ampicillin in pharmaceutical preparations, based on the copper facilitated formation of the stable acid degradation products, for which the presence of the intact antibiotic molecule is essential.

We have now adapted the method to the assay of ampicillin in chicken blood, bile