An acetylcholine-like effect of 2′(3-dimethylaminopropylthio)cinnamanilide

SIR,—The anti-5-hydroxytryptamine substance 2′(3-dimethylaminopropylthio)cinnamanilide (or Squibb compound 10,643), has been shown to be highly specific on the isolated rat uterus preparation (Rubin, Piala & others, 1964). It also has an anti-5-HT action on the bioluminescence of *Meganyctiphanes norvegica* (Doyle, 1966), but it has an acetylcholine-like effect on the isolated heart of the mollusc, *Mya arenaria.*

Work at the Squibb laboratories showed that this antagonist had about the same anti-5-HT activity as lysergic acid diethylamide on the isolated rat uterus and did not inhibit acetylcholine contractions in doses which were much larger than those sufficient to block the effect of 5-HT (Krapcho, Rubin & others, 1963; Rubin & others, 1964).

Recent work in this laboratory has shown that compound 10,643 has an acetylcholine-like effect on the isolated heart preparation of *Mya arenaria.* This mollusc heart responds to 5-HT by an increase in frequency and an increase in amplitude of heart beat, with sometimes an increase in tone. Acetylcholine causes a depression of the heart beat and a relaxation of the heart. Similarly Squibb 10,643 causes a depression of the heart beat and a relaxation of the heart. All these effects are easily removed by flushing the isolated organ bath with fresh isotonic solution or with filtered sea water. Doses of 5-HT and of Squibb 10,643 can be found which separately cause stimulation and depression, but which, given together, have no resultant effect on the recorded heart beat.

Concentrations of atropine which decrease the great sensitivity of the oestrous rat uterus preparation to acetylcholine fail to modify the sensitivity of the uterus to 5-HT (Amin, Crawford & Gaddum, 1954). Atropine has no anti-acetylcholine effect on the mollusc heart. Benzoquinonium chloride (Luduena & Brown, 1952) is an antagonist of the effect of added acetylcholine on several mollusc heart preparations, including that of *Mya arenaria.* Stimulation of the cardiac nerve of the clam, *Venus mercenaria,* during perfusion of the heart with benzoquinonium chloride caused acceleration of the heart beat, showing a blocking of the depressive action of acetylcholine released at the nerve endings (Welsh, 1953).

It has been reported that compound 10,643 inhibits the effects of 5-HT endogenously produced after the administration of its precursor, 5-hydroxytryptophan, in rats and mice, because it antagonizes the development of gastric erosions in rats and the cerebral excitation found in mice after the dosing of these animals with 5-hydroxytryptophan (5-HTP) (Rubin & others, 1964). Heart muscle of the mollusc, *Venus mercenaria,* has been shown to be capable of decarboxylating 5-HTP to 5-HT (Welsh & Moorhead, 1959). It is therefore possible that, in the heart preparation of *Mya arenaria,* the acetylcholine-like effect of this anti-5-HT compound is an unmasking of the action of an intrinsic acetylcholine-like substance by inhibition of the conversion of endogenous 5-HTP to 5-HT. But it must not be forgotten that the compound 10,643 can also counteract added 5-HT as in the rat isolated uterus preparation, so it may not, on the *Mya* heart be interfering with 5-HT metabolism, but simply with the action of already formed endogenous 5-HT.

Acknowledgements. Compound 10,643, was a gift from the Squibb Institute for Medical Research, New Brunswick, U.S.A. I thank the boat crews of the Marine Station, Millport for collecting the animals used.

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June 29, 1967

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Release of [^{3}H]noradrenaline from vasoconstrictor nerves

SIR,—The release of noradrenaline by nerve impulses from vasoconstrictor nerves is of great cardiovascular significance. This has been examined in the perfused hind limb of the dog (Rosell, Kopin & Axelrod, 1963) and more extensively in the cat spleen (see reviews by Brown, 1965; Gillespie, 1966). However, these experiments are complicated by the presence of non-vascular smooth muscle and concurrent fluctuations of perfusion parameters during nerve stimulation.

We have investigated the noradrenaline release in the structurally simple rabbit pulmonary artery which is adrenergically innervated (Bevan & Su, 1964; Verity & Bevan, 1966). This artery was cut spirally into a strip as small as 3 × 40 mm, weighing 40 mg. It was connected under a 2 g tension to an isometric strain gauge transducer, and mounted in a 2 ml tissue bath containing Krebs solution at 37° which was constantly stirred by bubbles of oxygen 95% and carbon dioxide 5%. The intramural adrenergic nerves were stimulated by a 2 min train of square wave impulses (0·3–1 msec duration, 10 cycles/sec, near maximal voltage), using platinum wire electrodes placed on either side of the strip. The contraction following stimulation was registered on a pen recorder.

The artery strip was initially incubated in Krebs solution containing 5 μc/ml (0·486 μM) of [(±)-7-^{3}H-noradrenaline]* hydrochloride (specific activity 10·28 c/mmole) for 30 min. This medium was then flushed and plain Krebs solution introduced into the tissue bath at a constant rate (1–2 ml/min). The overflow was collected in 1 ml aliquots for assay of tritium activity by scintillation spectrometry.

Thirty min after commencement of washing out, the first period of nerve stimulation was applied, and this elicited a sharp rise in the tritium outflow and muscle contraction (Fig. 1). Both responses returned to the baseline levels within 20 min. At this interval, stimulations were repeated up to 14 times with consistent contractile responses and a constantly diminishing but significant rise in tritium outflow. The first period of stimulation brought about a disproportionately great tritium output as compared to subsequent stimulations. Thus, in Fig. 1, the first peak represented a total of 93·0 nc tritium output, whereas the second, third and fourth peaks amounted only to 19·4, 11·8 and 9·9 nc, respectively. It is possible that some [^{3}H]noradrenaline was initially present either in the extracellular space or in an easily releasable form and was expelled by the first period of stimulation. The [^{3}H]noradrenaline

* 2-Amino-1-(1,3-dihydroxyphenyl)-[1-^{3}H]ethanol.