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Reversal by trypsin of the action of a 2-haloalkylamine

N-(2-bromo-ethyl)-*N*-ethyl-1-naphthalenemethylamine hydrobromide (SY28) is a potent member of a group of compounds, the 2-haloalkylamines, which are notable for producing an insurmountable antagonism to the motor action of noradrenaline on smooth muscle (Nickerson & Goodman, 1946; Graham, 1962). This blockade has been attributed to alkylation of the specific α -receptor (Harvey & Nickerson, 1954) and the nature of the bonding has been the subject of long research and much speculation (Belleau, 1958, 1959). In 1966 Graham & Katib described how addition of trypsin to a bath containing an isolated vas deferens of the guinea-pig, in which insurmountable antagonism to added noradrenaline had been produced by prior exposure of the tissue to a fully blocking dose of one of three 2-haloalkylamine compounds, reversed the blocking action. An attempt to repeat this work on rabbit vas has been reported (Moran, May & others, 1967) to have given equivocal results. Accordingly, the experiment has been repeated. Vasa from 400 g guinea-pigs were suspended in 10 ml of Hukovic solution at 37°, gassed with 5% carbon dioxide in oxygen, six together at a time, and stimulated 5 times with addition of noradrenaline 10^{-8} g/ml. ^{14}C -SY28 was then added to the bath for 20 min in a concentration of 1.34×10^{-6} containing 3.35×10^{-4} mCi in the 10 ml. Insurmountable antagonism to noradrenaline was then demonstrated, the tissue washed 12 times at 3 min intervals

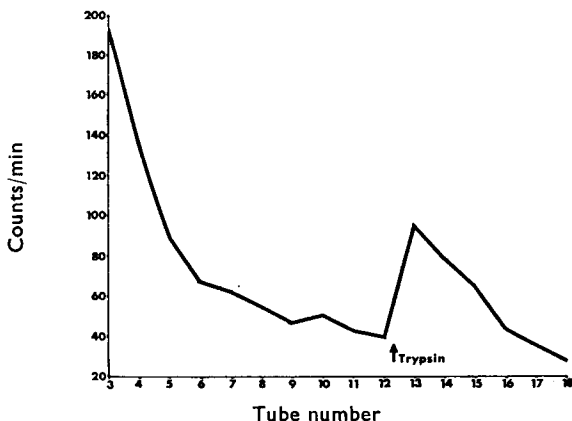


FIG. 1. The scintillation count in counts/min in the fluid in which was suspended guinea-pig vas deferens exposed to ^{14}C -SY28 1.34×10^{-6} in 10 ml for 20 min. Each tube no. refers to one 3 min cycle and a change of bath fluid. Trypsin 2.5×10^{-3} BAEE units/ml was added for 2 min at arrow.

and the ^{14}C -activity in aliquots of wash fluid determined by standard scintillation counting technique. The loss of ^{14}C -label from the tissue was by then at a steady and low level. Trypsin (Koch-Light) was added for 2 min at a concentration of 2.5×10^3 BAEE units/ml and washed out. A further six washes were collected and the output of ^{14}C -label determined in each. As may be seen in Fig. 1 the treatment with trypsin caused an increased output of specific activity. This episode coincided in time with the partial restoration of the response of the muscle to noradrenaline. The washes immediately before and after trypsin were concentrated at 50° by passing a warm air draught over the surface and run for 8 h on Whatman 3 MM paper in butanol:glacial acetic acid:water 50:12:25 as solvent. The front was marked and the dried paper exposed to Ilford Industrial G X-ray film in the dark for three months. The spot on the paper corresponding to radio activity on the film was ninhydrin positive, Rf 24.2. This result is interpreted to mean that suitable treatment with trypsin removes from guinea-pig vas deferens previously treated with SY28 a small peptide to which the 2-haloalkylamine is bound and that this restores its lost sensitivity to noradrenaline, possibly by uncovering an undamaged noradrenaline - receptor which was previously occluded by the blocking drug. Species differences may well require variation in the detail of enzyme treatment.

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The effects of stress and intravenous 0.9% NaCl injection on concentrations of whole brain 5-hydroxytryptamine in the neonate chick

Alterations in amounts of brain biogenic amines can occur under certain conditions of stress. Barchas & Freedman (1963) reported elevation of 5-hydroxytryptamine (5-HT) and lowering of noradrenaline in the brain of rats subjected to cold and swimming stress. Similarly, Goldberg & Salama (1969) observed an increase in rat brain 5-HT after stress with either electric shock or a revolving drum, although cold exposure alone did not produce this alteration.

The newly hatched chick and the newborn guinea-pig are functionally competent in the first hours of life. These two species do show a significant difference in that the guinea-pig has a well developed blood-brain barrier at birth, whereas the neonate chick does not and is considered to be neurologically immature (Waelsch, 1955; Lajtha, 1957; Key & Marley, 1962). Both however, are capable of surviving some forms of stress.

The basis of our present report occurred, initially, with the observation that one day old chicks entered a continuous roosting state when subjected to the stress of plucking neck feathers, making a small incision in the skin, or injecting 0.9% NaCl into the jugular vein. This was in contrast to the behaviour of untreated birds which