The occurrence of catecholamines in the sea anemone *Actinia equina*

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Ostland (1954) in surveying the distribution of catecholamines in invertebrates concluded that adrenaline, noradrenaline and dopamine were not present in the three coelenterates examined, which included the sea anemone *Metridium dianthus*. He claimed, however, that there was an unknown catecholamine present, which he called "Catechol 4." Dahl, Falk, Von Mecklenberg & Myhrberg (1963) using the formaldehyde fluorescence microscopy technique demonstrated the presence of a fluorescent substance resembling noradrenaline in cells and fibres of the ectodermal nerve net of the tentacles in the anemones *Metridium senile* and *Taelia felina*. No such fluorescence was found in any other part of the anemone.

In the present work the pattern of fluorescence distribution in *Actinia equina* has been found to be similar to that described by Dahl *et al.* (1963) for *Metridium senile* and *Taelia felina*.

Anemones were extracted with perchloric acid and the amines adsorbed on to alumina, eluted and separated by ion exchange or partition paper or thin-layer chromatography. Spots resembling dopamine and DOPA were detected. However, this pattern was seen in only eight of nineteen experiments. In eleven experiments no spots corresponding to known catecholamines were seen, but biologically inactive spots of high R_F value were observed. No spot resembling "Catechol 4" was seen. The reason for these two patterns of distribution of spots was found in the observation that many batches of anemones contained a substance which interfered with the normal travel of catecholamines on chromatographic media. This unidentified substance was removed by suitable ion exchange chromatography of the alumina extract, after which clear identification and separation of DOPA, dopamine and noradrenaline in extracts of *Actinia equina* was obtained. Identification was based on behaviour in chromatographic, fluorometric and biological tests. The concentrations of these three amines in whole anemones have been found to be low; DOPA 17 ± 4 ng/g, dopamine $14{\cdot}1 \pm 5{\cdot}5$ ng/g and noradrenaline $4{\cdot}7 \pm 0{\cdot}6$ ng/g (mean $\pm$ S.E. of eight experiments). Adrenaline, if present, occurs at a concentration of $< 0{\cdot}2$ ng/g. As expected from the fluorescence microscopy studies, the bulk of the noradrenaline is found to be present in the tentacles.

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Evidence for a molecular change in acetylcholine receptors produced by agonists

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Drug antagonism was studied using isometric contractions of thin strips of chick biventer cervicis muscle, suspended in Krebs solution at 37° C and stimulated with carbachol or suxamethonium. Tubocurarine and gallamine both behaved as conventional competitive antagonists, with dissociation equilibrium constants of $3{\cdot}78 \times 10^{-7}$M and $4{\cdot}70 \times 10^{-7}$M respectively.

Although decamethonium (C_{10}) stimulates chick biventer muscle, increasing N-substitution with alkyl or aryl groups results in the appearance of antagonist properties (Thesleff & Unna, 1954). Two such antagonists are decamethylene 1,10-*bis*-dimethylbenzyl-ammonium bromide (DPC_{10}), and decamethylene 1,10-*bis*-dimethyl (1-naphthylmethylene) ammonium bromide (DNC_{10}). The 2-chloroethyl derivative of DPC_{10}, decamethylene 1-(N-benzyl-2-chloroethylamino)-10-dimethyl-benzylammonium chloride hydrochloride, bears the same relationship to DPC_{10} as does benzilylcholine mustard (BCM) to benzilylcholine (Gill & Rang, 1966), and may accordingly be termed $DPC_{10}M$. Like other 2-haloalkylamines, $DPC_{10}M$ undergoes cyclization in solution to yield an ethyleniminium ion. It was hoped that, as with dibenamine and BCM, this might irreversibly alkylate receptors.

$DPC_{10}M$ did indeed produce an irreversible type of antagonism to carbachol and suxamethonium. The following observations suggested that $DPC_{10}M$ acted specifically on acetylcholine receptors: (*a*) contractions produced by 3·9 mM caffeine were unaffected by a concentration of $DPC_{10}M$ sufficient to abolish the response to a large dose of carbachol; (*b*) application of tubocurarine together with $DPC_{10}M$ prevented the appearance of the long-lasting $DPC_{10}M$ block. In early experiments the amount of antagonism produced by $DPC_{10}M$ appeared to be highly variable. The explanation of this variability was found to be that, in contrast to the action of BCM, the degree of block produced by $DPC_{10}M$ depended not only on the concentration of the antagonist and the time for which it was applied, but also on whether agonist was applied concurrently: rather than acting as "protecting" agents, agonists were found markedly to increase the blocking action of $DPC_{10}M$. Thus $1{\cdot}1\times10^{-6}$M $DPC_{10}M$ (as ethyleniminium) applied on its own for 15 min produced a final dose ratio to carbachol less than 1·2; when 3×10^{-5}M carbachol was applied for 4 min during the exposure to $DPC_{10}M$, the dose ratio produced was 2·0. This enhancement was seen even when $1{\cdot}5\times10^{-4}$M carbachol, applied for 90 sec, preceded the application of $DPC_{10}M$ by as long as 15 min. It is argued that this effect is due to the agonist causing a change in the receptors, the $DPC_{10}M$ having a greater affinity for receptors in this altered state than it has for those in the unchanged state. This has been termed a metaphilic effect of agonists, denoting their ability to alter the affinity of receptors for other compounds.

DPC_{10} and DNC_{10} were reversible antagonists, but they showed an equivalent phenomenon, the metaphilic effect appearing as "enhanced desensitization" in the presence of antagonist (see Flacke & Yeoh, 1968).

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The quantitative estimation of the activity of fourteen analogues of the neurohypophysial hormones on strips of mammary gland *in vitro*

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Quantitative estimation of the milk-ejecting activity of neurohypophysial hormones and analogue polypeptides is generally done *in situ* (for review see Berde & Bois-