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Search for catecholamine receptors

CONSIDERABLE progress has been made recently in the identification of membrane-bound receptor structures for polypeptide hormones such as insulin, glucagon and corticotrophin in mammalian tissues and cholinergic receptors in the electric organs of fish and eels. These studies have been based on the use of radioactive hormones or antagonists which bind very selectively to the receptor sites. Such binding if it is to be a valid method for identifying the receptors should show a very strict specificity, it should be saturable and indicate a finite and usually small number of receptor sites, and the affinity and rate constants for the binding reaction should correlate with the known biological properties of the receptor stimulant or antagonist molecule used. These criteria have been fulfilled in the instances just cited, and similar approaches have been successfully used to identify cholinergic muscarinic binding sites in mammalian brain, glycine receptor sites in spinal cord and receptors for morphine and other opiate drugs in brain (Hiley *et al., Biochem. J.*, **127**, 86P; 1972; Young and Snyder, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2832; 1973; Pert and Snyder, *Science, N.Y.*, **179**, 1011; 1973).

Cuatrecasas and colleagues, however, in last week's issue of *Nature*, (**247**, 92; 1974), illustrate the many pitfalls that await the unwary in using this apparently simple experimental approach for identifying receptor structures. Tritium-labelled noradrenaline of high specific activity has been available for some years, and might seem to offer a simple approach for labelling and identifying catecholamine receptors in mammalian tissues. Indeed Lefkowitz and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 1773; 1971; *J. biol. Chem.*, **248**, 342; 1973) have claimed that the binding of ^{3}H-noradrenaline to fragments of cell membrane in microsomal fractions from mammalian heart and liver represents a specific binding to β adrenoreceptors in these tissues. Cuatrecasas *et al.* have carefully assessed this claim and their article clearly refutes it.

The binding of labelled noradrenaline to membrane fragments from liver, heart and fat cells or to intact fat cells was saturable, but the number of binding sites present in fat cells was three to four orders of magnitude greater than the number of insulin or glucagon receptor sites measured previously in these cells. Furthermore, the binding of ^{3}H-noradrenaline did not obey the strict specificity that would be predicted if this binding did represent labelling of β adrenoreceptors. For example, the non-radioactive (+) and (−) stereoisomers of noradrenaline had identical affinities as competitive antagonists of ^{3}H-noradrenaline binding. The affinity constant for such inhibition was more than 1μM, whereas the apparent affinity constant of (−) noradrenaline at β adrenoreceptors in fat cells was less than 0.1 μM, and (+) noradrenaline was at least one thousand times less potent in eliciting β-adrenoreceptor responses. ^{3}H-noradrenaline binding was also antagonised quite effectively by various catechol compounds, including pyrogallol and catechol acids, none of which have any activity at all as β-adrenoceptor stimulants, nor do they antagonise the receptor actions of (−) noradrenaline or isoprenaline. On the other hand, neither the non-catechol compound soterenol, which is a potent stimulant of β adrenoreceptors, nor the β-adrenoreceptor antagonist drug propranolol had any marked inhibitory effects on the binding of ^{3}H-noradrenaline.

It is thus clear that the structure responsible for the membrane binding of ^{3}H-noradrenaline is not identical with the β receptor for catecholamines. Indeed the specificity of these binding sites for catechols and their sensitivity to known inhibitors of the enzyme catechol-O-methyl transferase (pyrogallol, tropolone, quercetin) lead Cuatrecasas *et al.* to conclude that these binding sites more probably represent a membrane-bound form of this enzyme, known to exist in the microsomal fractions of the tissues studied.

The use of labelled hormones and neurotransmitters or their antagonists is likely to remain a most fruitful approach to identifying elusive membrane-bound receptor sites. But since such receptors are present in most tissues in extremely small numbers such potential labels must bind with extremely high affinity, must be available with extremely high specific activity and must obey very precise specificity rules in order for this approach to be valid. The hunting of the receptor snark can otherwise all to easily lead to boojums.

From our Neuropharmacology Correspondent

Selective adhesion and retinotectal specificity

from a Correspondent

THE development of the brain involves the segregation of vast numbers of cells into precisely delineated subpopulations which are linked by highly selective neuronal connections. The mechanisms responsible for the formation of specific nerve connections have been analysed, most extensively in the retinotectal system of lower vertebrates: the axons of retinal ganglion cells connect selectively at local tectal sites to produce a map of the retina across the surface of the optic tectum. The most popular hypothesis that seeks to account for the discrimination in intercellular recognition that such selective connections imply is that due to Roger Sperry, who suggested, some 30 years ago, that cellular differentiation in the nervous system extends beyond the level of neuronal populations to that of the individual neurones themselves. The differentiated neurone acquires a characteristic cell surface label which participates in selective intercellular recognition and the formation of synaptic connections.

This hypothesis of neuronal specificity has withstood the test of time with remarkable success. Recent work has concentrated not so much on the hypothesis itself as on the rules by which such labelled neuronal arrays interconnect. The experimental paradigm adopted to examine the rules involves the surgical creation of relative size disparities between the innervating cell population and its target. Thus the patterns of connection that result when half a retina is caused to innervate a whole optic tectum or, conversely, when a whole retina feeds into a tectum half of which has been removed, have been described. The interpretation of such results has, however, run into a conceptual problem. One does not know whether the altered pattern of connections that is frequently found reflects altered neuronal labels, occurring as the direct result of experimental interference with a neuronal population or whether, alternatively, the neuronal labels themselves are unchanged by the operation. In this second case, the altered connection pattern might indicate the presence of a competitive mechanism in the formation of synaptic connections which are, then, determined not solely by the 'specificity labels' the neurones carry but also by the context in which the two extant neuronal populations are interconnecting (Gaze and Keating, *Nature*, **237**, 375; 1972). The inability to distinguish between these two alternatives will persist until the appearance of an experimental design in which it is possible to deduce the nature of the neuronal labels indepen-