The binding of benzyl alcohol to erythrocyte membranes

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Many studies of "non-specific" interactions of lipid soluble molecules with membranes have emphasized the correlation of biological activity (for example anaesthetic action) with oil/water partition coefficients. There is, however, considerable evidence that the orientation of an extraneous molecule in the membrane, its relation to the interface with the aqueous medium, and its distribution between the lipid and protein components, depends on both the structure of the small molecule and the precise organization of the membrane components.

We have found that the interaction of an anaesthetic, benzyl alcohol, with the erythrocyte membrane provides a simple illustration of the way in which interaction depends on the membrane structure. The partition coefficient for binding of benzyl alcohol to erythrocyte membranes is nearly constant at prelytic concentrations up to 80 mM, but shows a marked increase at higher concentrations in the lytic range. Pretreatment of the membranes with benzyl alcohol concentrations in the prelytic range has no effect on the partition coefficient and the interaction is reversible. However, pretreatment at the maximum concentration (300 mM) results in an irreversible increase in partition coefficient over the whole concentration range. The reason for this is quite clear when the binding to the separated membrane components is examined. The partition coefficient of the protein is approximately twice that of the membrane lipids; and the lipid value is itself greater than that of the intact membrane. The mean value of the separated components weighted according to the membrane composition is close to the value for membranes pretreated with 300 mM benzyl alcohol.

We conclude that in the intact membrane, the membrane structure itself restricts and modifies the interaction with benzyl alcohol. In the lytic range, the membrane progressively interacts as the sum of its separated components, and the major binding component is the membrane protein. It is also clear that these partition measurements on membrane preparations are more informative than partition coefficients measured in unrelated hydrophobic solvents.

Change from agonism to antagonism in the action of some oxytocin analogues: interpretation by a receptor model

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[2-O-Methyltyrosine]-oxytocin (methyloxytocin) may act on the rat or rabbit uterus *in vitro* as a full agonist, partial agonist, or antagonist according to the experimental conditions (Krejčí, Poláček & Rudinger, 1967). Other analogues of oxytocin modified in sequence position 2 (Zhuze, Jošt, Kasafírek & Rudinger, 1964) show similar properties to varying degrees and other tissues such as the isolated rat tail artery (Krejčí, Kupková & Vávra, unpublished results) react in a similar fashion.

The analogues on the one hand and the biological preparations on the other form graded series in their tendency to exhibit, or reveal, antagonism. On the isolated uterus low calcium concentration, no magnesium and a low temperature all favour the appearance of antagonism.

To explain the transition from agonism to antagonism on the same organ preparation with changing experimental conditions, a model is proposed in which methyloxytocin and related analogues are assumed to be partial agonists with respect to stimulus generation. Stimulus is here defined as that primary process initiating the response which results immediately from peptide-receptor interaction. The system is assumed to have a threshold and a receptor reserve. It is proposed that the experimental variables affect the efficiency of stimulus-response coupling. The conditions favouring antagonism also decrease sensitivity to oxytocin and can plausibly be assumed to interfere with the efficiency of stimulus-response coupling.

In conditions of high sensitivity there is an adequate receptor reserve and the stimulus generated even by methyloxytocin at full receptor occupancy is sufficient to elicit the maximal response. When stimulus-response coupling is made less efficient by changing conditions, part or all of the receptor reserve must be mobilized to provide the additional stimulus now required for maximal response. In the absence of an (adequate) receptor reserve the partial agonist character of methyloxytocin is now apparent also in the experimentally determined dose-response relations. As stimulus-response coupling deteriorates the threshold also rises and eventually the maximal stimulus generated by methyloxytocin is below threshold. The analogue becomes an antagonist.

It is suggested that the relation of the peptide to the receptor remains the same throughout. Binding of the hormone to the receptor is by non-covalent bonds only and there is evidence that magnesium, in addition to its effect on stimulus-response coupling, may potentiate binding at one particular (unidentified) site of interaction.

This model suggests: (1) the possibility of eliminating receptor reserves by utilizing them under changed experimental conditions; (2) the use of partial agonists for probing the processes linking stimulus with response; and (3) the choice of conditions suitable for the detection of functional deficiency in structural analogues.

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