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SHORT COMMUNICATIONS

Tryptophan inhibition of the blockade of a 2-halogenoalkylamine

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THE 2-HALOGENOALKYLAMINE group of compounds are powerful alkylating agents which bind irreversibly to the alpha adrenergic receptor, but which are also capable of alkylating other receptor sites.[1]

A number of suggestions have been made as to the chemical nature of the site of binding for 2-halogenoalkylamines, which includes proteolipid[2–4] and protein.[5,6] Graham and Mottram[6] found that, following exposure of guinea-pig *vas deferens* to the 2-halogenoalkylamine SY28, subsequent hydrolysis of the exposed tissue resulted in the isolation of a number of SY28-amino-acid complexes, which provides strong evidence to suggest protein is a principal binding site for 2-halogenoalkylamines. The chemical nature of the amino-acids involved in these complexes suggests binding occurs between the alkyl chain of the 2-halogenoalkylamine and nucleophilic groups located in the side-chains of the amino-acids involved.

It must be remembered however, that in drug–receptor interactions, bonding occurs through a number of sub-sites within the receptor. In the case of the alpha receptor, the binding of agonists and antagonists is greatly facilitated by the inclusion in the structural formulae of at least one aromatic group. Therefore we may assume that an aromatic sub-site forms an integral part of the alpha adrenergic receptor site.[7] It has even been suggested that there may even be more than one aromatic sub-site in the alpha receptor to cater separately for agonist and antagonist attachment.[8,9]

It was therefore decided to study the effects of amino-acids, containing an aromatic side-chain, on the irreversible blocking activity of the 2-halogenoalkylamine, *N*-(2-bromoethyl)-*N*-ethyl-1-naphthylmethylamine (SY28). This was achieved by measuring the degree of irreversible blockade against noradrenaline, produced by SY28, in the presence or absence of selected amino-acids.

Stripped guinea-pig *vas deferens*[10] were set up and bathed in Hukovics solution.[11] Control *vasa* were exposed to 10^{-8} g/ml SY28, a dose which produces a 90 per cent irreversible blockade of noradrenaline in this tissue. Test *vasa* were exposed to the same dose of SY28, but in the presence of varying doses of (−)-isomers of the aminoacids histidine, phenylephrine, tryptophan and tyrosine. In all cases 20 min was allowed for the development of the irreversible blockade of SY28. Results were measured by calculating the

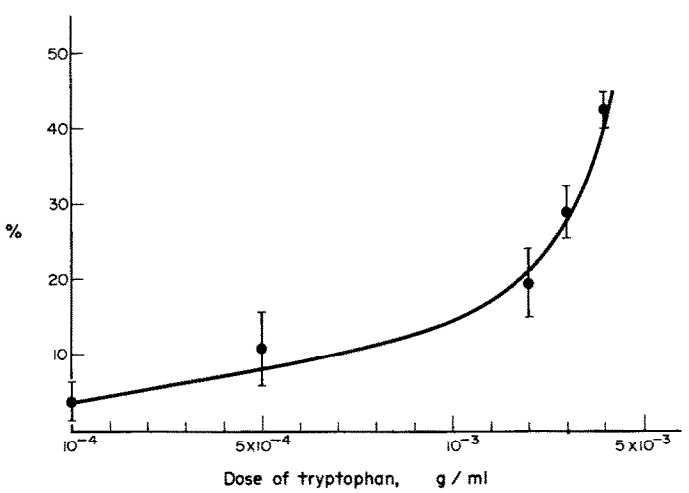

FIG. 1. Graph showing the effect of l-tryptophan in reducing the irreversible blockade of SY28 in guinea-pig *vas deferens*. The percentage reduction in SY28 blockade is plotted against increasing doses of l-tryptophan. Each point is the mean of four experiments ± S.E.

percentage reduction in responses to a standard dose of noradrenaline given before and after 20 min exposure to SY28 (with and without varying doses of amino-acid).

Results showed that the amino-acids, histidine, phenylephrine and tyrosine, exerted some slight degree of protection against blockade by SY28, but the only significant result was the dose-dependent effect of the amino-acid tryptophan. As the dose of tryptophan was increased, the degree of irreversible blockade by SY28 against noradrenaline was decreased (Fig. 1). The low solubility of (−)-tryptophan prevented experimentation using doses in excess of 4×10^{-3} g/ml.

There are two possible explanations to account for the action of tryptophan under these conditions. The amino-acid may be able to occupy the aromatic sub-site, normally occupied by SY28, within the alpha receptor and therefore compete with SY28 for that site. The other possibility is that tryptophan provides an alternative to the aromatic sub-site of the alpha receptor, therefore SY28 is preferentially bound to tryptophan, rather than the alpha receptor, when sufficient molecules of the amino-acid are available in the bathing fluid. Interaction between ^{14}C-SY28 and amino-acids, including to a small extent tryptophan, has been shown *in vitro*.[6]

It is therefore tempting to speculate that, assuming the alpha receptor is proteinaceous in nature, for which there is considerable evidence, the aromatic site within the alpha receptor, to which SY28 binds, is in fact tryptophan.

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Evidence for a peripheral effect of fusaric acid, a dopamine β-hydroxylase inhibitor, on serotonin metabolism

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FUSARIC acid (5-butylpicolinic acid) has been shown to be a potent inhibitor of dopamine-β-hydroxylase (DBH)[1]. Johnson *et al.* have described another group of DBH inhibitors, the most studied being 1-phenyl-3-(2-thiazolyl)-2-thiourea (U-14,624)[2].

Besides depleting the brain norepinephrine (NE) stores, these two inhibitors influence the brain 5-hydroxytryptamine (5-HT) metabolism, increasing the cerebral levels of tryptophan (TRY) and 5-hydroxyindole acetic acid (5-HIAA) with a slighter elevation of 5-HT itself.