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Binding sites for 2-haloalkylamines

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The dibenamine group of drugs, of which SY28 (N-(2-bromo-ethyl)-N-ethyl-N_1-naphthylmethylamine HBr) is an active member, may occupy specific or nonspecific (silent) receptors or binding sites; it has been suggested that one of these is protein (Takagi & Takahashi, 1968). Support for this contention was provided by Graham & Katib (1966), who found that α-adrenoceptor blockade in guinea-pig vas deferens could be removed by treatment with trypsin. At the same time there was a release of labelled haloalkylamine (Mottram & Graham, 1970). Attempts have been made to isolate drug-protein complexes as follows. Stripped vasa from guinea-pigs of 350–450 g weight were exposed to ^{14}C-SY28 (10^{-6} g/ml; $6{\cdot}7\times10^{-5}$ mCi/ml) for 20 min at 37° C in gassed Hukovic's solution. They were washed three times and extracted for 2 min at 70° C with 10 ml of chloroform : methanol (2 : 1), with agitation. The residue was roughly homogenized in a Tri-R homogenizer with a glass mortar and teflon pestle, an ice bath being used. The residue was spun down at 2,500 *g* for 10 min, resuspended in 10 ml/g of phosphate buffer of pH 6·5 and cysteine HCl (5×10^{-4} g/ml) and papain (0·1 ml, B.D.H.) added. The digest after 36 h at 60° C was hydrolysed in 6 N HCl at 110° C for 18 h. Ascending paper chromatography (Whatman 3MM paper with butanol : acetic acid : water 50 : 12 : 25 as solvent for 8 h) followed by autoradiography for approximately 90 days with Ilford Industrial G X-ray film revealed that the labelled SY28 was bound. The papain digest consistently gave three spots, the R_f values of which varied with the extent of digest. The hydrolysates gave three consistent spots apart from the spot due to unbound ^{14}C-SY28. The acid hydrolysates were passed through 30 cm columns of cationic (50×2) and anionic (1×2) Dowex resins, and Sephadex G10 gel, the effluents being collected in 2 ml aliquots. The concentration of isotopic label in the samples was measured by standard scintillation counting technique. Those samples which contained high activity were subjected to repeat paper chromatography and autoradiography as described above. This produced four spots of consistent R_f values, as shown in Table 1. It is concluded that a haloalkylamine (SY28) may be recovered by papain

TABLE 1. *Rf values with standard deviations from acid hydrolysates before and after purification*

Before	After
32·5±0·8	33·0±0·4
40·0±0·9	40·4±0·9
44·3±1·2	43·9±0·5
	49·6±0·9
85·8±1·5	85·3±1·4

digestion and acid hydrolysis from smooth muscle to which it has been bound and that the complexes are to amino-acids.

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Effects of McN-A-343 on responses induced by sympathetic nerve stimulation in the rabbit isolated ear artery

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Acetylcholine and other cholinomimetic drugs affect the responses of isolated arterial preparations to sympathetic nerve stimulation. The responses may be enhanced or decreased depending on the frequency of stimulation and the concentration of drug (Malik & Ling, 1969a; Rand & Varma, 1970). Both of these effects are produced by the purely nicotinically acting drug DMPP (Malik & Ling, 1969b). We have investigated the effects of McN-A-343 [4(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride; *m*-$Cl.C_6H_4.NH.CO.O.CH_2.C:C.CH_2.N^+(CH_3)_3.Cl^-$), which was reported to stimulate only mucarinic receptors of ganglion cells (Roszkowski, 1961).

With low frequencies of stimulation (<5 Hz), vasoconstrictor responses induced by sympathetic nerve stimulation were reduced by infusion of McN-A-343, but the vasoconstrictor effects of noradrenaline were not affected. In the presence of atropine, McN-A-343 did not reduce responses induced by sympathetic nerve stimulation. Similar effects were observed with methacholine. These results suggest that cholinomimetic drugs may act muscarinically to impair the release of noradrenaline by sympathetic nerve impulses. Direct evidence for such an effect of acetylcholine has been produced by Löffelholz & Muscholl (1969). Amphetamine reversed the impairment of sympathetic nerve stimulation by acetylcholine (Malik & Ling, 1969a), McN-A-343 and methacholine, indicating a similarity between their effects and that of adrenergic neurone blocking drugs.

Enhancement of the effects of sympathetic nerve stimulation was produced by infusions of McN-A-343 when high frequencies of stimulation (>10 Hz) were used or when low frequencies were used in the presence of atropine. With high frequencies of stimulation, the responses were gradually decreased after prolonged infusion of high concentrations of McN-A-343 (2 μg/ml). In the presence of atropine, the effects of McN-A-343 in producing at first an increase and then a decrease in responses induced by high frequencies of stimulation were not altered qualitatively, but the effective concentrations were higher than in the absence of atropine. The enhancement of responses elicited by sympathetic nerve stimulation by McN-A-343 suggests either facilitation of noradrenaline release or blockade of reuptake of noradrenaline; since the effects of noradrenaline were only slightly increased, both factors may be involved. The secondary decrease may be due to reduction of transmitter reserve with a high frequency of stimulation.

Our interpretation of the findings is that McN-A-343 acts muscarinically on the adrenergic terminal axons innervating the rabbit ear artery to impair or to enhance