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PROTEOLIPID AS A BINDING SITE FOR 2-HALOALKYLAMINES

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Abstract—Vasa of guinea-pigs were incubated with a 2-halogenoalkylamine [^{14}C]SY28 30 μM, 25 mc/m-mole, before or during exposure to 3·65 mM phentolamine, extracted with 2:1 chloroform–methanol and the extracts washed. The organic (lower) phase was subjected to hydrolysis with 6 N HCl and autoradiographs of paper chromatographs made. Three spots (in addition to the parent compound) were demonstrated in extracts of controls and two spots from phentolamine-treated tissue. SY28–amino acid complexes were compared as to R_f values and selected candidates for the binding site are discussed.

AXELROD, Aronow and Brodie[1,2] studied the uptake of dibenamine and phenoxybenzamine into fat depots in dogs. They suggested that lipid is a storage site from which these drugs are slowly released, thus accounting for the long duration which is a feature of their action. It has been shown[3] that the irreversible action of these drugs is preceded by the formation of an ethyleneimminium ion; covalent binding to the receptor site must follow, and the extent of the blocking action is proportional to the amount of ion present. Fiszer and DeRobertis[4,5] studied the binding of labelled SY28 and dibenamine in subcellular fractions from basal ganglia and brain stem of the cat and rat. SY28 is a synthetic 2-halogenoalkylamine which is similar in its properties to phenoxybenzamine.[6] They found the highest uptake of these drugs was in the fractions containing nerve-ending membranes. The [^{14}C]SY28 was readily extracted from the synaptosome-containing fraction with chloroform–methanol (2:1, v/v) and, after water partition, most of the isotope count remained in the organic phase or total lipid extract. They suggested that the alpha receptor may be a special proteolipid mainly found in membranes. The present study is concerned with the binding of [^{14}C]SY28 to the smooth muscle cells of the vas deferens of the guinea-pig, and its recovery from cell fractions.

METHODS

Guinea-pigs weighing 350–450 g were stunned, bled and the vasa immediately removed and stripped of their mesenteric investment.[7] The tissue was washed in saline and incubated with [^{14}C]SY28, 30 μM (specific activity 25 mc/m-mole in the carbon atom of the $-\overset{|}{N}-\overset{*}{C}H_2$ group) at 37° for 30 min, and washed with saline every 5 min, for 1 hr. Extraction was carried out in chloroform–methanol (2:1, v/v) at 37° for 45 min. The extracts were then washed with 20% by vol. of water then twice with

the theoretical upper phase of Folch, Lees and Sloane Stanley.[8] The lower phase, which contains proteolipids and some phospholipids, was retained, evaporated almost to dryness at 70° and acid hydrolysis carried out with 6 N HCl in sealed tubes at 110° for 24 hr. The resultant hydrolysates were run on Whatman 3MM chromatography paper with butanol–acetic acid–water (50:12:25, by vol.) as solvent for 8 hr. The front was marked and the papers dried and exposed to Ilford Industrial G X-ray film for periods up to 3 months.

In some experiments the tissue was pretreated for 10 min with 3·65 mM phentolamine mesylate before adding [^{14}C]SY28 without washing. Alanine, aspartic acid, glutamic acid, serine, histidine and methionine were mixed with [^{14}C]SY28 in equimolar amounts and run on the same paper in the same solvent, and autoradiographs were prepared as above. The R_f values of the resulting spots were noted.

RESULTS AND DISCUSSION

Figure 1 shows the autoradiograph from the chromatograph of the acid hydrolysates from control (*C*) and phentolamine pre-treated (*P*) washed lipid extracts. Most of the material is unbound [^{14}C]SY28, R_f 86·9 ± 0·8 seen near the solvent front. In control experiments three other radioactive spots are seen, in extracts of phentolamine pre-treated vasa only two. The R_f values of all radioactive spots are given in Table 1.

Acid hydrolysis of this severity may be relied upon to break down the proteolipids in this extract to individual amino acids. Amino acid candidates for the observed radioactive complexes have been selected by trial. No amino acid has been found to complex with [^{14}C]SY28 to give a spot of R_f 52·1 ± 0·4. This spot may be due to the labelled drug binding to some non amino acid site such as phosphate.

TABLE 1

Phentolamine treated	Control	Amino acid candidate
86·8 ± 1·0	86·9 ± 0·8	Unbound SY28
51·8 ± 0·5	52·1 ± 0·4	—
—	43·5 ± 1·3	Alanine, aspartic acid, glutamic acid, serine
38·7 ± 0·5	38·9 ± 1·0	Histidine, methionine

$\%R_f$ values (mean ± S.D.) of autoradiographic spots from chromatographs of hydrolysate of lipid extracts of guinea-pig was 3·65 mM incubated with [^{14}C]SY28 30 μM; control and pretreated with phentolamine and amino acids which complex with SY28 and give the same R_f value. The number of experiments for each value is 4.

Serine is a candidate for the spot of R_f value 43·5 ± 1·3. A possible source of this amino acid could be proteolipid or phosphatidylserine. Alanine, aspartic and glutamic acids also make complexes which have the same R_f.

The "alpha receptor" blocking agent phentolamine reduces the intensity of binding of [^{14}C]SY28 to all of these complexes and the spot of R_f 43·5 ± 1·3 is abolished. This may indicate that one or all of these amino acids is an integral part of a binding site which involves the "alpha receptor". Dikstein and Sulman[9,10] have suggested that a phospholipid (possibly phosphatidylserine) may be involved in the alpha receptor site in rabbit aorta, and DeRobertis and Fiszer[4,5] postulated a "special" proteolipid

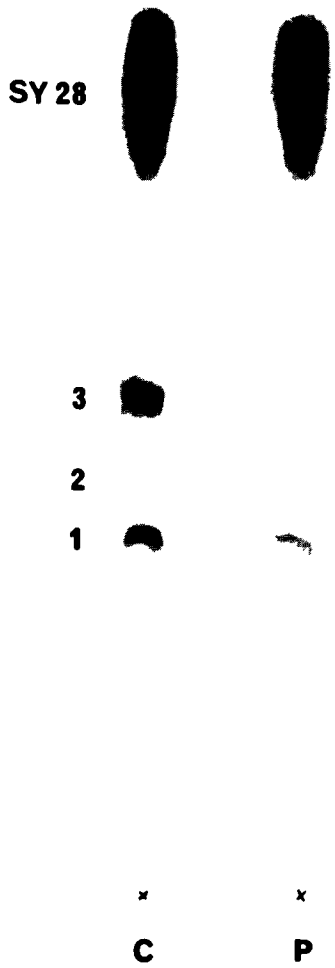

FIG. 1. Autoradiograph of paper chromatograph of acid hydrolysates of washed lipid extracts from guinea-pig vasa incubated with [^{14}C]SY28, control (*C*) and phentolamine pre-treated (*P*). The radioactive spots are designated 1, 2 and 3. Note the absence of 2 in the extract of vas pre-treated with phentolamine.

for neurones. The results presented in this paper confirm that 2-halogenoalklyamines complex with proteolipid fractions in smooth muscle and that the antagonist drug phentolamine can mask a constituent of this complex which may include one or more of four amino acids.

SUMMARY

(1) Groups of vasa deferentia from guinea-pigs were incubated with [^{14}C]SY28 30 μM before or during exposure to 3·65 mM phentolamine.

(2) A 2:1 chloroform–methanol extract was made from each group, washed, and the lower phase hydrolysed with 6 N HCl.

(3) Autoradiographs were prepared from paper chromatographs of the hydrolysates and the R_f values of the three spots obtained were noted. Two identical spots were located from extracts of phentolamine treated vasa; one was no longer present.

(4) Six selected amino acids were complexed under similar conditions with [^{14}C]SY28 chromatographs run under identical conditions and autoradiographs prepared. The R_f values were noted.

(5) Several amino acid candidates as binding sites for SY28 are postulated. The possibility of serine being a constituent of the alpha receptor is discussed.

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