Isoprenaline relaxed the longitudinal muscle of guinea-pig colon bathed with Krebs solution at 32° C. Reproducible dose-response curves were obtained for isoprenaline in the dose-range 0·3–300 ng/ml. Propranolol was added to the bathing fluid for a contact period of 45 min. With low concentrations (1–10 ng/ml) of propranolol the dose-response curve for isoprenaline was shifted to the right by up to 30-fold, but these shifts were not always dose-dependent. Moreover, higher concentrations (10–125 ng/ml) of propranolol had little further blocking effect. Similar results were obtained using coaxially-stimulated ileum or longitudinal strips of oesophageal muscle from the guinea pig, or when the β-adrenoceptor blocking drugs sotalol or practolol were used instead of propranolol.

Ahlquist & Levy (1959) showed that inhibitory effects of catecholamines in intestine are mediated *via* both α- and β-adrenoceptors. Therefore, the interaction of isoprenaline with the irreversible α-adrenoceptor blocking drug dibenamine was investigated. In guinea-pig colon exposed to dibenamine (10 μg/ml) for 30 min, the response to isoprenaline was either completely abolished or the dose-response curve was shifted to the right and the maximum relaxation attainable was greatly reduced. In only two of seven dibenamine-treated preparations was the residual response to isoprenaline large enough for the interaction with propranolol to be examined. In these preparations responses to isoprenaline were progressively blocked by propranolol (5, 25, 125 ng/ml).

Bartlet & Hassan (1969) showed that chick rectum contained β- but not α-receptors. Isoprenaline relaxed this preparation in the dose-range 0·3–30 ng/ml. The dose-response curve for isoprenaline was progressively shifted to the right by increasing concentrations of propranolol (5, 25 and 125 ng/ml). This interaction clearly differed from that in guinea-pig colon.

The results, which confirm those of Farmer & Levy (1969), show that in guinea-pig colon the relaxation induced by isoprenaline is mediated mainly through stimulation of α-adrenoceptors. Consequently, propranolol and other β-blocking drugs have little blocking action. In chick rectum, where isoprenaline-induced relaxation is mediated *via* β-adrenoceptors, propranolol exerts its normal blocking action.

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Relative affinities of some α-adrenoceptor blocking drugs in isolated human smooth muscle

I. M. COUPAR* and P. TURNER, *Division of Clinical Pharmacology, Medical Professorial Unit, St. Bartholomew's Hospital, London, E.C.*1

Information on new drugs is often derived only from studies in animal tissues.

Although responses of isolated tissues from animals and man are usually the same, information derived from human tissue should also be available whenever possible. Human tissue has, therefore, been used to assay the potency of a number of α-adrenoceptor blocking drugs. Previous work (Coupar & Turner, 1969) has shown that isolated human ileum and saphenous vein respond reproducibly to noradrenaline and might, therefore, be useful preparations for screening new α-adrenoceptor blocking drugs.

Fresh surgical specimens were prepared for isotonic recording as described previously (Coupar & Turner, 1969). The affinity of α-adrenoceptor antagonists was assessed on ileum by measuring the pA_2 values against noradrenaline (Schild, 1947) determined after a 2 min contact time (Lockett & Bartlet, 1956).

The results using ileum showed that the affinity for α-adrenoceptors was phentol-

TABLE 1. *pA_2 values of α-adrenoceptor blocking drugs measured on human ileum (longitudinal muscle) and saphenous vein (circular muscle)*

	Number of determinations	Range	Mean
Ileum			
Phentolamine	4	(6·80–7·20)	7·0
Thymoxamine	4	(6·72–6·96)	6·9
Azapetine	4	(6·22–6.81)	6·5
Clonidine	3	(5·5–7·7)	5·6
Tolazoline	4	(4·80–5·16)	4·9
Vein			
Clonidine	3	(6·45–7·25)	7·0

pA_2 values were determined at 2 min on ileum. Strips of vein were incubated with clonidine for 10 min before cumulative noradrenaline contractions were produced. The pA_2s were then calculated from the dose ratios. The Krebs bicarbonate solution contained amechol (100–250 ng/ml) for the determinations on ileum.

amine≏thymoxamine, azapetine, clonidine, tolazoline in decreasing order (Table 1). As clonidine (ST 155) is undergoing clinical trial as an antihypertensive agent and its action has been said not to involve α-adrenoceptor blockade (Zaimis & Hanington, 1969) its effect was investigated on saphenous vein as an example of vascular smooth muscle. 10 min incubations with clonidine caused parallel shifts to the right of noradrenaline dose response curves, and the pA_2 values calculated from the dose ratios (Schild, 1957) were more than one unit higher than on ileum. This discrepancy was not due to the different contact times used since the pA_2 value of clonidine on ileum was the same when measured at 2 and at 10 min. These results support the view that clonidine may have an action on vascular smooth muscle independent of receptor blockade (Zaimis & Hanington, 1969). Work is in progress to determine if the pA_2 values of thymoxamine on vein are similar to those on ileum as thymoxamine is known to be a competitive and relatively specific α-adrenoceptor blocking drug on animal tissues (Birmingham & Szolcsànyi, 1965) and on isolated human arteries (Birmingham, Ernest & Newcombe, 1969).

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Binding sites for 2-haloalkylamines

D. R. MOTTRAM (introduced by J. D. P. GRAHAM), *Department of Pharmacology, Welsh National School of Medicine, The Parade, Cardiff*

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.