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THE EFFECT OF 2-HALOGENOALKYLAMINES ON THE BIOLOGICAL ACTIVITY OF SOME PEPTIDES

BY

J. D. P. GRAHAM AND H. AL KATIB

From the Department of Pharmacology, Welsh National School of Medicine, Cardiff

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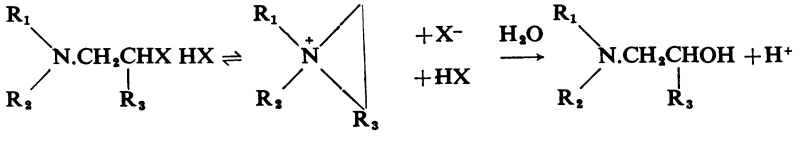
Rocha e Silva, Corrado & Ramos (1960) showed that pre-injected phenoxybenzamine greatly prolongs the hypotensive action of bradykinin in the cat and Rocha e Silva & Leme (1963) further showed that dibenamine and phenoxybenzamine first potentiate and subsequently antagonize the stimulant action of this peptide on isolated guinea-pig ileum. A noradrenaline-blocking dose of 10 mg/kg does not reduce the pressor response to injected vasopressin in the fowl (Harvey, Copen, Eskelson, Graff, Poulsen & Rasmussen, 1954), but 120 mg/kg of *N*-2-chlorethyl-*N*-ethyl-*N*-1-naphthylmethylamine, an active compound, abolished it in two fowls. Harvey & Nickerson (1954) reported that the pressor activity of vasopressin in the cat was reduced if it was incubated with dibenamine for 24 hr before injection of the mixture. Konzett (1960) in a brief note mentioned that pre-added dibenamine has little or no influence on uterine contractions induced *in vitro* by oxytocin and Graham (1959) failed to demonstrate any effect by pre-treatment of Xenopus adapted to light on a white background (LA/WB) with these blocking drugs on the increase in melanophore index brought about by injection of β-melanophore stimulating hormone (MSH) from pig. In view of the well documented instability of 2-halogenoalkylamines in buffered aqueous solution, it was thought desirable to check whether these effects are functions of the ethyleneiminium ion (E^+) [or where relevant the carbonium ion (C^+)] which is responsible for blockade of the noradrenaline-sensitive alpha receptors, or of the ethanolamine which is inactive in the latter respect. The chemical transformations which produce these species in aqueous solution may be indicated thus:



(1) parent compound (2) ethyleneiminium ion (E^+) (3) ethanolamine
(In dibenamine, X=Cl; in SY28 and L_2, X=Br)

The opportunity was also taken to extend the range of compounds tested in order to determine whether or not these effects are general for 2-halogenoalkylamines and to use compounds which exhibit a wide range of potency as antagonists of noradrenaline *in vivo* in order to observe the degree of correlation between this and the effect on the activities of the peptides. The 2-halogenoalkylamine compounds selected were

N-2-chlorethyldibenzylamine hydrochloride (dibenamine); *N*-(2-bromoethyl)-*N*-ethyl-1-napthalene methylamine hydrobromide (SY28) and 2-bromo-2-phenyl-ethyl dimethylamine hydrobromide (L_2, Graham & James, 1961) and their synthetic hydrolysis products, the ethanolamines. In one experiment, the ethyleneiminium ion of an extremely potent compound [2-(3-iodophenyl)-2-bromo] ethyl dimethylamine (No. 11 of Graham & Karrar, 1963) was used, as a picrylsulphonate. These compounds are known to exhibit a rate of cyclization to E^+, speed of onset of antagonism and potency as antagonists of noradrenaline which is in the order dibenamine<SY28<L_2, whereas the duration of the block is in the reverse order, dibenamine>SY28>L_2.

METHODS

Bradykinin (freeze-dried samples from two sources, see acknowledgements) was freshly dissolved in water for each test, as were all the compounds except dibenamine and SY28, which were freshly dissolved in a concentration of 1:1,000 w/v in acidified 70% w/v alcohol/water before dilution in saline.

(a) A control dose-response curve for bradykinin was established at 37° C in Huković's (1961) solution on stripped isolated vas deferens from guinea-pigs weighing 250 to 350 g; on the same piece of tissue a fresh dose-response curve was established after pre-treatment with various concentrations of 2-halogenoalkylamines or ethanolamine for various periods of time.

(b) On other pieces of vas deferens a dose-response curve to bradykinin was established in order to determine the maximum and a dose selected which produced a 40% response. This was applied for 45 sec every 5 min before and after various concentrations of the three halogenoalkylamines and the ethanolamines.

(c) The isolated vas deferens was stimulated with a submaximal concentration of bradykinin. Dibenamine (10 mg as the hydrochloride) or an equimolar amount of SY28 (as the hydrobromide) or of L_2 (as the hydrobromide) or their ethanolamine analogues were incubated at 37° C for 15 min with 1 mg of bradykinin in acid-water of *p*H 5, in which the formation of ethanolamine from the parent compound is inhibited. The bradykinin was thus kept for the stated time with (1) halogenoalkylamine and (2) with ethanolamine, before dilution, neutralization and performance of a dose-response curve.

(d) The sensitivity of guinea-pig ileum to bradykinin was determined before and after treatment with the E^+ (as a picrylsulphonate) of [2-(3-iodophenyl)-2-bromo] ethyl dimethylamine (No. 11 of Graham & Karrar, 1963) added for 100 sec contact time. A similar experiment was carried out using the E^+ form of compound 11 (9.5 mg) incubated for 15 min with 1 mg bradykinin (1) at *p*H 5 (formation of ethanolamine inhibited), (2) at *p*H 9 (formation of ethanolamine encouraged) and (3) as a control 1 mg bradykinin was incubated with an equivalent amount of sodium picrylsulphonate at *p*H 7.

(e) A piece of vas deferens was treated 5 times with a submaximal dose of bradykinin, washed every 10 min for 3 hr, exposed to ethanolamine and further washing carried out. The effect of repeated washing on the bradykinin-ethanolamine-tissue relation was thus explored.

Oxytocin (Syntocin, Parke Davis & Co.) of known structure was used throughout all tests.

(a) 5 ml. solution containing 10 u./ml. was incubated for 24 hr at 37° C with 29.6 mg dibenamine or equimolar SY28, L_2 or the three ethanolamines in 0.06 N solution of sodium bicarbonate (see Harvey & Nickerson, 1954) in which E^+ and ethanolamine are formed. Control oxytocin was treated in the same way without addition of the compound. The relative activity of the two solutions on the rat uterus was determined by the assay method of the British Pharmacopoeia, 1963. Each compound was tested on 10 uteri from different animals for each test.

(b) A submaximal concentration of oxytocin having been selected from a dose response curve its effect was measured before, during and after the addition of each of the three compounds and

three ethanolamines, allowing 20 min contact. A range of concentrations was used to determine ED50's and the time-course of washout and recovery studied. A fresh tissue was used for each test.

Argenine-Vasopressin (Pitressin, Parke Davis & Co) was used throughout the tests.

(a) 5 ml. solution containing 10 u./ml. was incubated at 37° C for 24 hr with 29.6 mg dibenamine or equimolar SY28, L_2 or the three ethanolamines in 0.06 N solution of sodium bicarbonate. Control vasopressin was treated in the same way without addition of compound. The relative activity of the two solutions was determined by the assay method of the British Pharmacopoeia, 1963, on rat blood pressure.

(b) A dose of 10 m-u. vasopressin was injected every 10 min in the above preparation before and for 3 hr after graded doses from 10 to 40 mg/kg of the three 2-halogenoalkylamines and the three alkylethanolamines.

Angiotensin (Hypertensin, Ciba Labs Ltd) in a dose of 0.5 or 1.0 μg/kg was injected at intervals into rats similarly prepared, before and after 2-halogenoalkylamine or ethanolamine, and after incubation of 1 mg with 2 mg dibenamine or equimolar amounts of the other compounds in 0.06 N solution of sodium bicarbonate, or saline free from compound, at 37° C for 3 and 24 hr. This experiment was repeated on isolated guinea-pig ileum with the six compounds added to the bath before angiotensin, or incubated with angiotensin and the mixture assayed.

Melanophore Stimulating Hormone (MSH). A purified pig β-MSH of known constitution (Karkun & Landgrebe, 1963) was assayed for MSH activity in Xenopus against international standard ox MSH. The pig β-MSH, which contained 1,200 u./mg material, was then diluted to 2 u./ml. and incubated by itself or with compound No. 11 of Graham & Karrar (1963) or with SY28 or SY28-ethanolamine, in water, 1 mg of material to 2 mg of compound, at 37° C for 1 hr and diluted. An appropriate dose (0.0013 u./toad) was administered into the dorsal sacs of groups of five Xenopus, light adapted on a white background and the melanophore index measured half-hourly for 2½ hr, on five occasions. Xenopus is sensitive to acid or alkaline injections.

L-*Arginine hydrochloride* was mixed in equimolar amounts with dibenamine, SY28 and L_2 and incubated for 10 min at 37° C in Huković's solution. The mixture was used to determine the ED100 of the halogenoalkylamine in blocking noradrenaline on isolated guinea-pig vas deferens compared with the ED100 without arginine.

RESULTS

Bradykinin. The contraction of isolated guinea-pig vas is potentiated by exposure to halogenoalkylamine or to ethanolamine for short periods (5 min). The order of potency of equimolar amounts of the parent compounds is L_2>SY28>dibenamine, which coincides with the rate of cyclization to E^+ and also with the rate of hydrolysis to ethanolamine. This relation is shown in Fig. 1,A. This finding corresponds with the initial potentiation of bradykinin on guinea-pig ileum by phenoxybenzamine described by Rocha e Silva & Leme (1963). If the duration of contact of the haloalkylamine with the tissue is increased to 20 min before bradykinin is applied, the three parent compounds cause a parallel shift of the linear log dose-response (as % maximum) to the left, i.e., potentiate, but the order of potency is now dibenamine>SY28>L_2 (see Fig. 2). This effect is also produced by the ethanolamines and in this same order of potency. When the parent haloalkylamines are left in contact for longer periods a progressive antagonism to the effect of bradykinin sets in (see Fig. 3). It is partially surmountable and reversible by washing after dibenamine (3.8×10^{-5}M), SY28 (6.8×10^{-6}M) or L_2 (4.3×10^{-6}M). Higher concentrations produce irreversible insurmountable block. This order of potency parallels the rate of onset and the potency for antagonism of noradrenaline (see Table 1). When

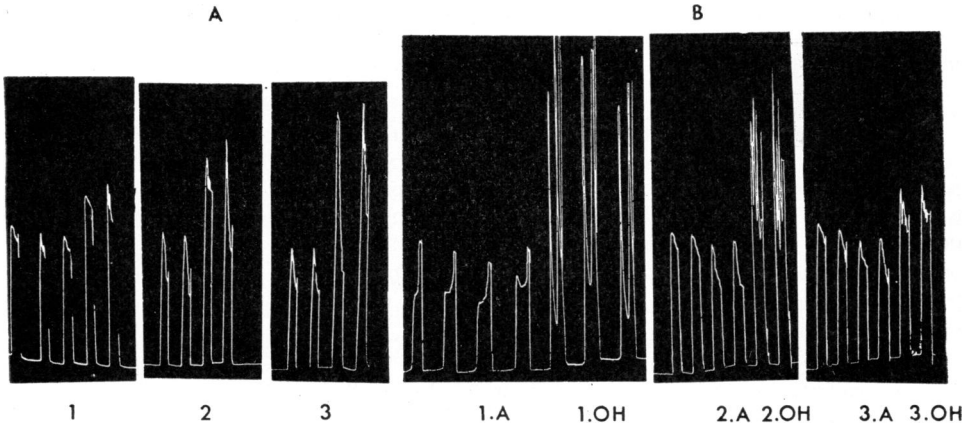


Fig. 1. Isolated guinea-pig vas deferens stimulated with bradykinin 0.3 mg/l. A, The potentiation of the response caused by 5 min exposure to (1) dibenamine; (2) SY28; (3) L_2, each 10^{-5}M. B, The potentiation of the response after incubation of the peptide with the ethanolamine (OH) but not the amine (A); (1), (2), (3) as in 1,A.

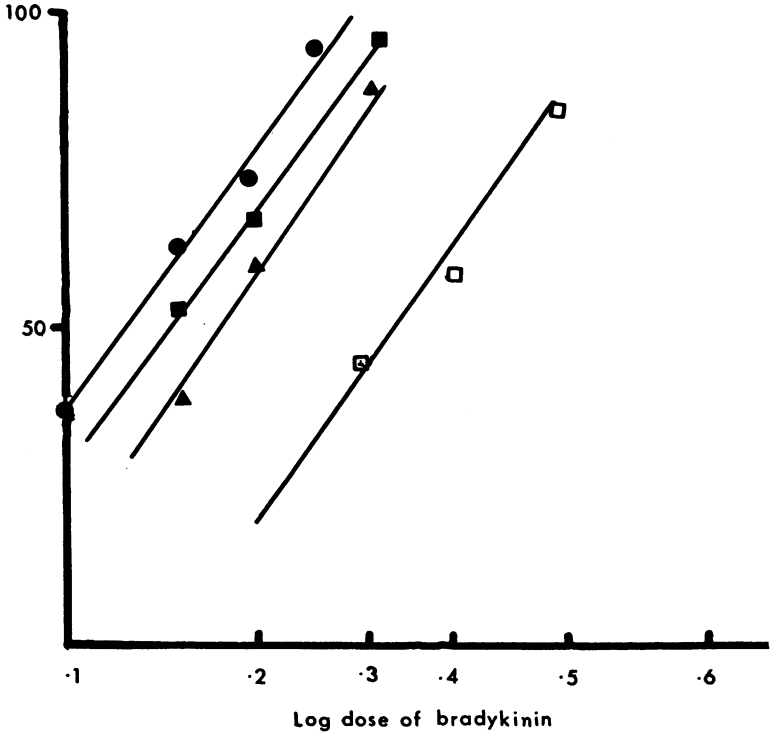


Fig. 2. Bradykinin on isolated guinea-pig vas deferens. The shift of the dose-response curve after 20 min exposure to three halogenoalkylamines. ●——●, dibenamine; ■——■, SY28; ▲——▲, L_2; □——□, control.

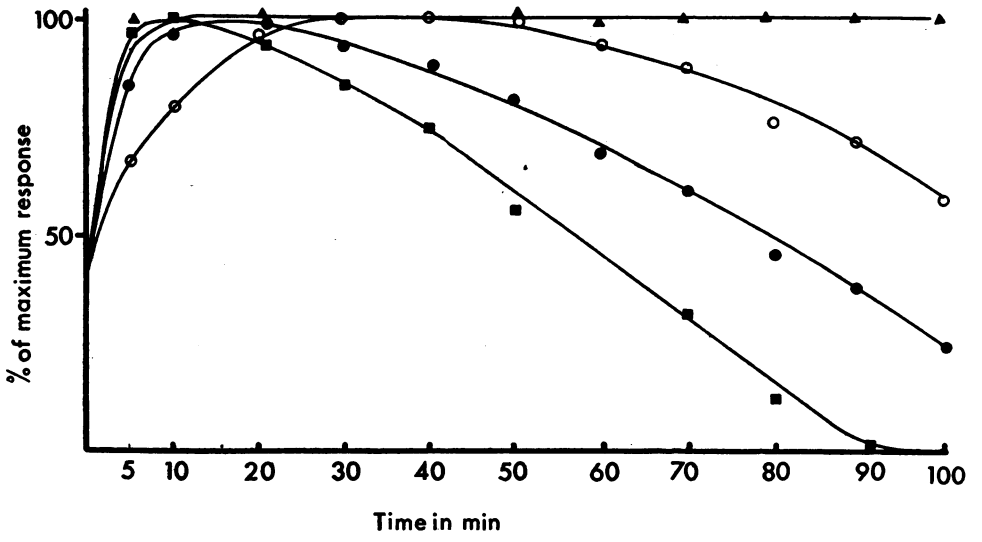


Fig. 3. Isolated guinea-pig vas deferens. The progressive antagonism to the action of bradykinin displayed by the parent 2-halogenoalkylamines. Contrast with the potentiation caused by the ethanolamines, for any length of contact. Dibenamine, ○——○; SY28, ●——●; L_2, ■——■; any of the ethanolamines, ▲——▲.

TABLE 1

THE EFFECT OF THREE HALOGENOALKYLAMINES AND THEIR ALKYLPHENYLETHANOLAMINES ON THE RESPONSE OF THE GUINEA PIG VAS TO BRADYKININ, AND ON THE PRESSOR EFFECT OF NORADRENALINE IN THE RAT (GRAHAM & AL KATIB, 1966)

Compound	Guinea-pig vas deferens		Rat pressor test	
	Time of maximum potentiation	Time to develop	ED50 in μ moles/kg	Time to develop full antagonism
Dibenamine	20–50 min	180 min	41	30–60 min
Dibenamine-OH	5 min	—	Inactive	—
SY28	10–20 min	130 min	25	10–15 min
SY28-OH	5 min	—	Inactive	—
L2	5–10 min	100 min	3	1 min
L2-OH	5 min	—	Inactive	—

the peptide is pre-incubated separately with the antagonists and the ethanolamines and then added to the vas deferens, the parent compound has no effect on the action of bradykinin but the ethanolamine potentiates (see Fig. 1,B). The order of potency is dibenamine-OH>SY28-OH>L_2-OH. When ethanolamine is applied to an isolated vas deferens it has no apparent effect, but if bradykinin is applied in the presence of ethanolamine, the action of the peptide is enhanced, but not if the ethanolamine is firstly well washed out. If, however, the bradykinin is applied first and even if it is well washed out the subsequent application of ethanolamine causes a repetitive spasm of the vas deferens. In this respect dibenamine-OH>SY28-OH>L_2-OH (see Fig. 4). This spasm is not abolished or prevented by atropine, hexamethonium, trasylol or added calcium ions but ceases after cortisol hemisuccinate 10 μg/ml. for 10 min or as soon as the vas

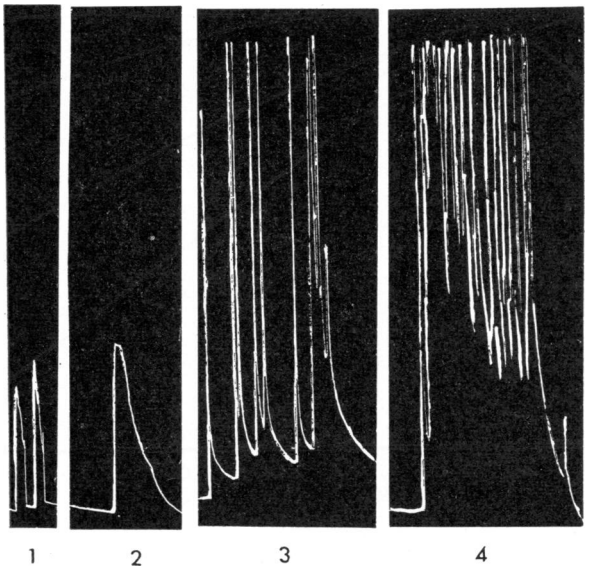


Fig. 4. Isolated guinea-pig vas deferens. The spasmogenic effect of the three ethanolamines applied at increasing intervals of time and washing after bradykinin. Dibenamine alcohol is the most active. 1, Bradykinin ; 2, L_2-OH ; 3, SY28-OH ; 4, Dibenamine-OH. Between 1 to 2, 2 to 3 and 3 to 4, 20 min wash.

deferens is washed. Furthermore the E^+ of [2-(3-iodophenyl)-2-bromo] ethyl dimethylamine (No. 11) in a dose which does not itself affect the ileum prevents the response to bradykinin if added to the bath before the peptide, but if incubated at *p*H 5 with bradykinin *in vitro* and the two added as a mixture, the response is not reduced. Its ethanolamine (produced by incubation in alkalinized saline) is a potentiator in either way of presenting it.

It follows, therefore, that the ethanolamine potentiates bradykinin on the vas deferens or the ileum of guinea-pig while the parent compound (E^+ or C^+), in concentrations that are 1,000 times higher than those needed to block alpha receptors for noradrenaline, abolishes the action of the peptide and the response to other agonists. Of the three ethanolamines examined, dibenamine-OH is the strongest potentiator, whereas of the parent compounds L_2 is the strongest antagonist (excluding the limited test on [2-(3-iodophenyl)-2-bromo] ethyl dimethylamine picrylsulphonate). Bradykinin must be very firmly bound to the tissue since the ethanolamine-bradykinin synergism is seen after several hr of repetitive washing, or it must induce a "permanent" sensitization of the tissue which makes the ethanolamine a powerful stimulant.

Oxytocin. Of the three parent compounds, only dibenamine exhibits an antagonism to oxytocin on isolated rat uterus, and that is weak and takes an hour to develop but does increase with time. The ED50 at 20 min is 2.3×10^{-6}M in the bath. The ethanolamines have no effect. When incubated as a mixture neither the parent compounds nor the ethanolamines modify the response to syntocin on rat uterus. The picture is one of absence of significant interaction between this peptide and these compounds.

Vasopressin. Neither of the three parent compounds in doses of 10 to 40 mg/kg (which is more than sufficient to abolish the pressor response to noradrenaline) modify the pressor response to pitressin in rats. For this reason pitressin has been used as a test of specificity in examining this type of compound. Higher doses are toxic and cause a failure of reactivity in the preparation for all agonists. The ethanolamines are inactive. When incubated mixtures are assayed, it is again found that the etholamines are inactive but the amines reduce the potency of the peptide (see Table 2). In this respect SY28>dibenamine>L_2.

TABLE 2

THE % REDUCTION OF PRESSOR RESPONSES IN THE RAT TO PITRESSIN WHEN THE PEPTIDE IS PRE-INCUBATED WITH 2-HALOGENOALKYLAMINE BUT NOT WHEN INJECTED SEPARATELY AND AFTER THE COMPOUND

Compound	Incubated mixture	Injection, not mixed
Dibenamine	32±5	0
Dibenamine-OH	0	0
SY28	65±5	0
SY28-OH	0	0
L2	30±5	0
L2-OH	0	0

Angiotensin. The pressor response in the rat to this peptide is not affected by prior injection of full doses of haloalkylamine or ethanolamine. Toxic amounts ultimately reduce the response. Incubating angiotensin 1 mg with haloalkylamine or ethanolamine 2 mg for 3 and 24 hr at 37° C does not modify the potency of the peptide. A similar lack of effect of the compounds is found when the test object for the angiotensin is isolated guinea-pig ileum.

Melanophore Stimulating Hormone (β-MSH). Injecting Xenopus toads with 2-halogenoalkylamines or with ethanolamine has no effect on the rate and degree of change of melanophore index, M.I. (a measure of MSH action), subsequently brought about by injection of a preparation of pig β-MSH. Injection of a pre-incubated mixture of β-MSH with E^+ or -OH does not inhibit the melanophore expanding activity (increase in M.1.).

These results are summarized in Table 3.

TABLE 3

Preparation	Separate injection		Mixture	
	E^+	-OH	E^+	-OH
Oxytocin rat uterus	0 or decr.	0	0	0
Vasopressin rat B. Pr.	0	0	decr.	0
Bradykinin guinea pig vas and ileum	decr.	incr.	0	incr.
Angiotensin rat B. Pr.	0	0	0	0
M.S.H. Xenopus melanophore	0	0	0	0

incr.=increase. decr.=decrease of activity. 0=no effect.
In the case of oxytocin only dibenamine is active; in the case of vasopressin and angiotensin all 3 parent compounds were tested; in the case of bradykinin and M.S.H. E^+ of compound 11 was used additionally.

L-*Arginine-haloalkylamine mixture.* Mixing L-arginine with parent compound inhibits or even inactivates it, as will any amino acid with which it reacts, e.g., cysteine, lysine, etc.

DISCUSSION

Rocha e Silva *et al.* (1960, 1963) attributed the potentiated response of smooth muscle to bradykinin caused by 2-halogenoalkylamines to inhibition of a hypothetical tissue kinase. It is now evident that the potentiator is not the parent compound but the ethanolamine (inactive against noradrenaline). It is improbable that this chemical species exerts its effect by inactivation of a kinase because one would expect such an enzyme to remove all traces of bradykinin from the tissue isolated in a bath for an hour whereas it can be seen in Fig. 4 that peptide is probably still present after that time. Bradykinin must be firmly bound to tissue in order to be thus released or activated by 2-haloalkylethanolamine after prolonged washing or it must bring about a " permanent " sensitization of the tissue in the sense that subsequent addition of ethanolamine acts as or releases a stimulant. The ethanolamine may facilitate the transport or access of bradykinin to its site of action, which is not the alpha receptor for noradrenaline. Nor is it likely that bradykinin releases noradrenaline, because ethanolamine does not potentiate the action of that amine and E^+ blocks it in 1,000 times less amount than is needed to block bradykinin. The spasm is not the result of a cholinergic mechanism nor is it due to lack of calcium. The concentration of imine (or carbonium) ion necessary to abolish responses to bradykinin is of the same order as that needed to abolish contractile responses to acetylcholine, or to reduce that to K^+, and is three logarithmic removes from that needed to abolish responses to noradrenaline (or histamine by SY28). It is probable, therefore, that such concentrations act upon a common pathway from the various receptors (for noradrenaline, dopamine, histamine, acetylcholine, bradykinin) to the initiation of contraction. But the ethanolamine must act upon a part of the mechanism " bradykinin-receptor occupation-contractile response," because it potentiates the response to this peptide but not to any other of those tested (see Table 3) nor to catecholamine, acetylcholine, histamine (Graham & Al Katib, unpublished).

The established structure of the peptides submitted to incubation with halogenoalkylamines and their ethanolamines is as follows:

	1	2	3	4	5	6	7	8	9	
Syntocin:	cyS.	tyr.	ileu.	glu(NH_2)	asp(NH_2)	cyS.	pro.	leu.	gly(NH_2)	(9)
Arg. vasopressin:	cyS.	tyr.	phe.	glu(NH_2)	asp(NH_2)	cyS.	pro.	arg.	gly(NH_2)	(9)
Bradykinin:	H-arg.	pro.	pro.	gly.	phe.	ser.	pro.	phe.	argOH	(9)
Angiotensin:	H-asp(NH_2)	arg.	val.	tyr.	val.	his.	pro.	pheOH		(8)
β-MSH:	H-asp. glu. gly. pro. tyr. lys. met. glu. his. phe. arg. tyr. gly. ser. pro. pro. lys. asp-OH									(18)

The main finding is that ethanolamine is a potent potentiator of bradykinin. The concentration of E^+ (a cationic N^+ or C^+) needed to abolish the response to bradykinin is so non-specific in its effects as to imply that the site of this action is not the bradykinin receptor but a point nearer to the contractile mechanism. Bradykinin contains free terminal arginine moieties which are important to its activity (Khairallah & Page, 1963). One might expect a potent E^+ to bond with the COO^- or NH_2 radicles of these amino acids ; free arginine *in vitro* does inactivate E^+. If E^+ bonds with these arginine moieties it certainly has no effect on the complementarity of the molecule to its receptor. Harvey & Nickerson (1954) suggested that the inhibitory effect of dibenamine when incubated with vasopressin is due to alkylation of amino nitrogen (NH_2), but this cannot be the whole effect because oxytocin (unaffected) and vasopressin (antagonized) equally contain

4-glu(NH_2) 5 asp(NH_2) and angiotensin (unaffected) contains terminal asp(NH_2). The structural difference lies in 3 ileu. and 8 leu. in oxytocin and 3 phe. 8 arg. in vasopressin. The pressor activity of both 8-arginine and 8-lysine vasopressin is destroyed after incubation with tryptic enzyme (Lawler & du Vigneaud, 1953) but not oxytocic activity in oxytocin. The pressor activity of vasopressin must therefore depend largely on the 8-arginine linkage which may be the site of alkylation by E^+. A highly basic amino acid in position 8 has been shown to be necessary for activity in vasopressin (Sawyer, 1961). The structural requirement for the stimulant effect of oxytocin on the uterus is not specific, because bradykinin, vasopressin, angiotensin and other peptides are equally effective on this tissue. β-MSH from pig is an open-ended octadecapeptide containing 5, 17-lys and 11-arg. Some specific activity is retained in this peptide when it is fragmented into a smaller molecule (Karkun & Landgrebe, 1963; Woolley & Merrifield, 1963), so that alkylation at these sites might occur without much loss of activity.

SUMMARY

1. Three 2-halogenoalkylamine compounds, viz: dibenamine, SY28 and L_2 (Graham & James, 1961) and the ethanolamines derived from them were investigated for their effects on bradykinin, oxytocin, vasopressin, angiotensin and melanophore stimulating hormone (β-MSH).

2. Ethanolamine potentiates bradykinin on guinea-pig vas deferens and the parent compound (or its imine, E^+; or carbonium, C^+; ion) in much higher concentrations antagonizes it. Dibenamine-OH is the most potent potentiator, L_2 the most potent antagonist.

3. It seems likely that the antagonism is non-specific in the sense that it is only accomplished by concentrations of E^+ which abolish or reduce the response of the vas to noradrenaline, histamine and acetylcholine.

4. No other peptide is affected so obviously, but dibenamine exerts an antagonism to oxytocin on isolated rat uterus.

5. If the peptides and the parent compounds are incubated together and then added to the preparations, ethanolamine still potentiates bradykinin but E^+ is unable to block this tissue before the bradykinin causes a full contraction.

6. The pressor effect of vasopressin in rat is reduced by pre-mixing and incubating the peptide with 2-halogenoalkylamine but not with ethanolamine.

7. The compound does not exert this effect by itself *in vivo*; it cannot therefore block the vasopressin receptor, nor can vasopressin act by releasing noradrenaline. The peptide must be alkylated, not solely at an NH_2 group (because oxytocin and angiotensin, which equally contain NH_2 groups, are not affected) but possibly at the 8-arginine.

8. MSH is not affected.

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REFERENCES

GRAHAM, J. D. P. (1959). Antagonism by 2-haloalkylamine compounds of some actions of adrenaline, noradrenaline and isoprenaline on Xenopus laevis. *Archs int. pharmacodyn Thér.*, **118**, 317–326.

GRAHAM, J. D. & JAMES, G. W. (1961). The pharmacology of a series of substituted 2-halogenoalkylamines. *J. mednl pharm. chem.*, **3**, 489–504.

GRAHAM, J. D. P. & KARRAR, M. A. (1963). Structure-action relations in N,N-dimethyl-2-halogenophenethylamines. *J. mednl pharm. chem.*, **6**, 103–107.

HARVEY, S. C. & NICKERSON, M. (1954). Reactions of dibenamine and some congeners with substances of biological interest in relation to the mechanism of adrenergic blockade. *J. pharmac. exp. Ther.*, **112**, 274–290.

HARVEY, S. C., COPEN, E. G., ESKELSON, D. W., GRAFF, S. R., POULSEN, L. D. & RASMUSSEN, D. L. (1954). Autonomic pharmacology of the chicken with particular reference to adrenergic blockade. *J. pharmac. exp. Ther.*, **112**, 8–22.

HUKOVIĆ, S. (1961). Responses of the isolated sympathetic nerve-ductus deferens preparation of the guinea pig. *Br. J. Pharmac. Chemother.*, **16**, 188–194.

KARKUN, J. N. & LANDGREBE, F. W. (1963). Pituitary hormones affecting the chromatophores. In *Comparative Endocrinology*, **1**, 81–110. Academic Press, N.Y.

KHAIRALLAH, P. A. & PAGE, I. H. (1963). Effects of bradykinin and angiotensin on smooth muscle. *Ann. N.Y. acad. Sci.*, **104**, 212–221.

KONZETT, H. (1960). Specific antagonism of dibenamine to ergometrine. In *Adrenergic Mechanisms a Symposium*, pp. 463–465. Churchill, London.

LAWLER, H. C. & DU VIGNEAUD, V. (1953), Enzymatic evidence for intrinsic oxytocic activity of the pressor-antidiuretic hormone. *Proc. Soc. exp. Biol. med.*, **84**, 114–116.

ROCHA E SILVA, M., CORRADO, A. P. & RAMOS, A. O. (1960). Potentiation of the duration of the vasodilator effect of bradykinin by sympatholytic drugs and by reserpine. *J. pharmac. exp. Ther.*, **128**, 217–226.

ROCHA E SILVA, M. & LEME, G. J. (1963). Antagonists of bradykinin. *Medna. exp.* (Basle), **8**, 287–295.

SAWYER, W. H. (1961). Neurohypophysial hormones. *Pharmac. rev.*, **13**, 225–277.

WOOLLEY, D. W. & MERRIFIELD, R. B. (1963). Anomalies of the structural specificity of peptides. *Ann. N.Y. acad. Sci.*, **104**, 161–171.