Biochemical Pharmacology, 1966, Vol. 15, pp. 1303–1308. Pergamon Press Ltd., Printed in Great Britain.

THE INHIBITION OF CATIONIC MYOTROPIC DRUGS BY COMPOUNDS 48/80 AND 46/108*

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(*Received* 27 *December* 1965; *accepted* 28 *March* 1966)

Abstract—The action of angiotensin, oxytocin, bradykinin, acetylcholine, and 5-hydroxytryptamine upon the isolated rat uterus was antagonized by the products of condensation of *p*-methoxyphenethyl-methylamine with formaldehyde. The inhibition was of the competitive reversible type and was observed both with compound 48/80 (a mixture of dimer, trimer, and higher polymers) and with compound 46/108 (the pure dimer), but the former was about twice as active on a weight basis, though slower in its action, than the latter. The contraction of the uterus produced by barium chloride was not inhibited by the largest concentrations of compounds 48/80 and 46/108 employed. The quantitative analysis of the inhibition by compound 46/108 was taken as evidence that the antagonist competes for an anionic site in the receptor that is common to the five agonists studied.

SMOOTH-muscle contraction can be produced by the action of several cationic drugs such as acetylcholine, 5-hydroxytryptamine, oxytocin, bradykinin, and angiotensin. In particular, these drugs produce contractions of the isolated rat uterus that are qualitatively identical, although there is a wide variation in their molar activity. This, and the fact that all these substances are positively charged molecules at the physiological pH, suggest that their cell-wall receptors may have a common feature, possibly an anionic binding site.

It has been suggested[1] that cell membrane acid polysaccharides might participate in the receptor mechanism involved in smooth-muscle response to cationic drugs, and heparin has been proposed as a model of the cell-surface polysaccharide. The binding of histamine[2] and angiotensin[3] by heparin *in vitro*, has been reported, and dyalysis equilibrium experiments have shown that the same is true of acetylcholin, hydroxytryptamine, oxytocin, and bradykinin.† The binding of all these drugs by heparin is competitively inhibited† by compound 48/80,[2, 3] a mixture of condensation products of *p*-methoxyphenethyl-methylamine with formaldehyde.[4]

As part of an attempt at better understanding the drug–receptor interaction of the myotropic activity of angiotensin and other cationic substances, we have investigated the inhibition of the response of the isolated rat uterus to some drugs by compounds 48/80 and 46/108. The latter is the pure dimer of *p*-methoxyphenethyl-methylamine with formaldehyde.

* This work was supported in part by U.S. Public Health Service Grant RF-87-01. and by the Fundação de Amparo à Pesquisa do Estado de São Paulo.

† Hampe, Teixeira, and Paiva; unpublished data.

MATERIAL AND METHODS

Compounds 48/80 and 46/108 were obtained from the Wellcome Research Laboratories, Tuckahoe, N.Y. Angiotensin was the synthetic Asp(NH_2)[1]-Val[5]-angiotensin II prepared by CIBA Ltd., Basel. Bradykin in and oxytocin were the synthetic peptides produced by Sandoz AG, Basel. Acetylcholine chloride was a product of Eastman Kodak, and 5-hydroxytryptamine creatinine sulfate complex, B grade, was purchased from the California Foundation for Biochemical Research.

The isolated rat uterus was prepared as described previously.[5] One uterine horn from a virgin rat previously treated with stilbestrol was suspended in de Jalon's physiological solution of the following composition per liter: NaCl, 8 g; KCl, 0·4 g; $CaCl_2$, 0·06 g; $MgCl_2$, 0·04 g; $NaH_2PO_4 \cdot H_2O$, 0·05 g; $NaHCO_3$, 1 g; glucose, 1 g. The contractions were registered with a frontal lever with 1·5-g load and twofold magnification. For each of the agonists studied the responses of the uterus to three concentrations were repeatedly recorded; the de Jalon's solution was then replaced as the bathing medium by a similar solution containing compound 48/80 or compound 46/108. The agonist continued to be administered regularly at 3-min intervals until a constant response was obtained and measured at three dose levels. A new de Jalon's solution containing an increased concentration of compound 48/80 or 46/108 was then substituted for the previous one and the process repeated. After dose–response curves were obtained in the presence of two to four concentrations of the antagonist, unmodified de Jalon's solution was used again as the bathing medium for observation of the recovery from inhibition.

RESULTS

When the physiological salt solution in which the uterus was suspended was replaced by a similar solution containing 0·1 mg compound 48/80 in 1 l., inhibition of the responses to angiotensin, bradykinin, oxytocin, acetylcholine, and 5-hydroxytryptamine was observed. This inhibition was developed slowly, reaching a maximum after 60–90 min, and could be reversed by resuspending the muscle in de Jalon's solution free of compound 48/80. The recovery from inhibition was also slow, but was complete after 60–120 min.

All five drugs studied were significantly inhibited by compound 48/80 at a concentration of 10^{-4} g/l. Figure 1 shows that in the presence of compound 48/80 the log dose–effect curves were displaced horizontally but without a significant change in the slope. The highest concentration of inhibitor that was used (4×10^{-4} g/l.) had no effect on the response of the uterus to barium chloride.

Compound 46/108 also antagonized the five drugs that were studied, without any effect on the response of the uterus to barium chloride. A typical inhibition is shown in Fig. 2. Compound 46/108 was about half as active an inhibitor as compound 48/80, on a weight basis. However, the antagonism with compound 46/108 was established more quickly, and after its removal the recovery from inhibition was also faster than that observed with compound 48/80. The inhibition reached its maximum 10–15 min after the uterus was put in contact with compound 46/108, and within 5–10 min after its removal the normal uninhibited response was re-established (Fig. 3).

Figure 4 shows the log dose–response curves for the five agonists in the presence of varying concentrations of compound 46/108. Since this is the pure dimer, with

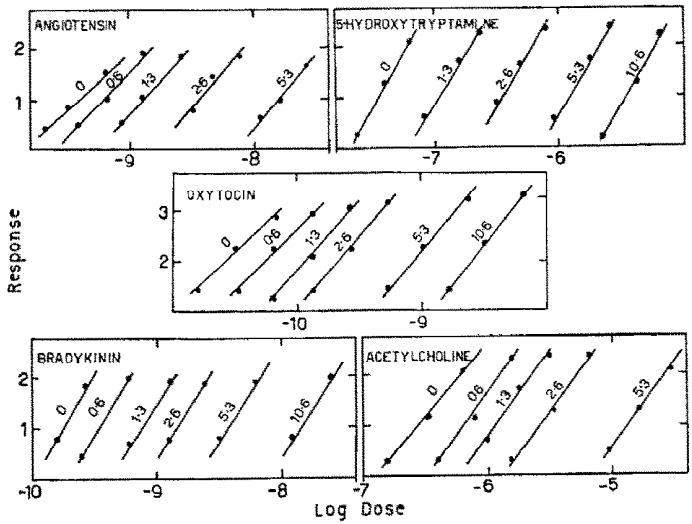

FIG. 1. The effect of compound 48/80 on the response of the isolated rat uterus to five cationic drugs. Doses of agonists in moles/l. and responses in cm of twice-amplified contraction records. The figures beside each line show the concentrations of compound 48/80, in g/l., $\times 10^4$.

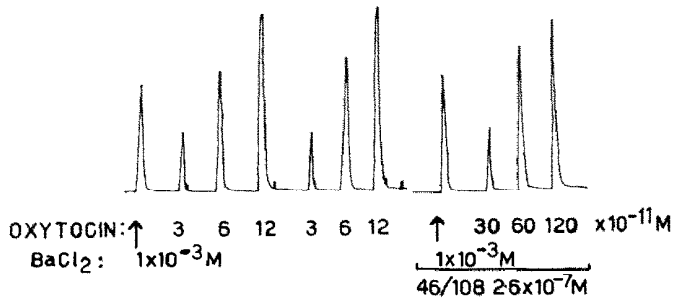

FIG. 2. The effect of compound 46/108 on the response of the isolated rat uterus to oxytocin and barium chloride. The concentrations of oxytocin, barium chloride, and compound 46/108 are given in moles/l. of bathing solution.

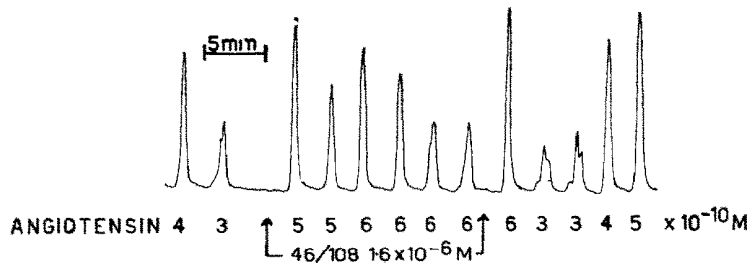

FIG. 3. The effect of compound 46/108 on the response of the isolated rat uterus to angiotensin. The recording of the contractions was obtained without stopping the kymograph drum. The concentrations of angiotensin and compound 46/108 are given in moles/l. of bathing solution.

known molecular weight, the concentrations are expressed on a molar basis. With this compound, as with the mixture contained in compound 48/80, a parallel displacement of the log dose–response curves was observed for the five agonists studied, without an apparent tendency toward decreasing slopes with the higher inhibitor concentrations.

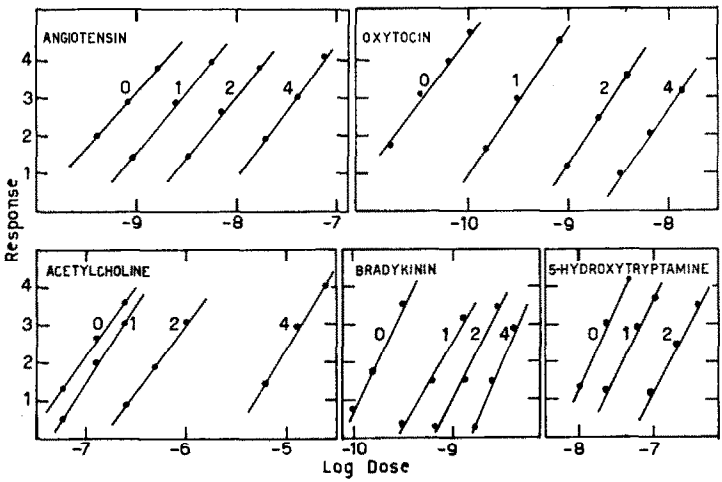

FIG. 4. The effect of compound 46/108 on the log dose–response curves of five myotropic substances on the isolated rat uterus. Doses of agonists in moles/l. and responses in cm of twice-magnified contraction record. The molar concentrations of compound 46/108 ($\times 10^6$) are shown beside the respective lines.

DISCUSSION

The products of the condensation of *p*-methoxyphenethyl-methylamine with formaldehyde were found to be effective inhibitors of the response of the isolated rat uterus to angiotensin, oxytocin, bradykinin, acetylcholine, and 5-hydroxytryptamine. No inhibition of the response to barium chloride was observed even with the highest concentrations of the antagonists that were employed. This indicates that the inhibition of the other five drugs is at the level of drug receptor interaction rather than at that of effector mechanisms.

Compounds 48/80 and 46/108 produced displacements of the log dose–effect curves that were not accompanied by significant changes of the slopes. This was observed even when the antagonist concentrations were sufficiently large to necessitate a 40- to 160-fold increase in the concentration of agonist to produce the same responses as in the uninhibited preparation. Such behavior is characteristic of reversible competitive inhibition.[6, 7]

For all five agonists that were studied, compound 48/80 was twice as active, although much slower in its inhibitory action, than compound 46/108. This indicates that one or more of the higher condensation products present in compound 48/80[4] is more active than the dimer in compound 46/108, but the latter seems to have an easier transit through the diffusion barriers, resulting in a faster attainment of equilibrium concentrations in the biophase. It is not possible to compare the activity of the different condensation products because of lack of information on the exact composition of compound 48/80. Also for this reason, the results obtained with compound 46/108 are more amenable to quantitative treatment and interpretation.

The data obtained with compound 46/108 were plotted according to the equation proposed by Arunlakshana and Schild:[8]

$$\log (x - 1) = \log K_B - n\mathrm{p}A_x \qquad (1)$$

where x is the ratio of doses necessary for producing the same response in the presence and in the absence of a certain concentration of antagonist, $\mathrm{p}A_x$ is the negative logarithm of the antagonist concentration, K_B is the association constant for the formation of the antagonist–receptor complex, and n is the number of moles of antagonists that bind to one "receptor site." This equation is derived from the assumption that the agonist–receptor interaction is bimolecular and that the molecules of antagonist combine with the receptor site by a reversible competitive mechanism.

The plots of log $(x - 1)$ vs. $\mathrm{p}A_x$ for the five agonists studied are shown in Fig. 5. Very similar straight lines were obtained, all with intercepts at the abscissae between

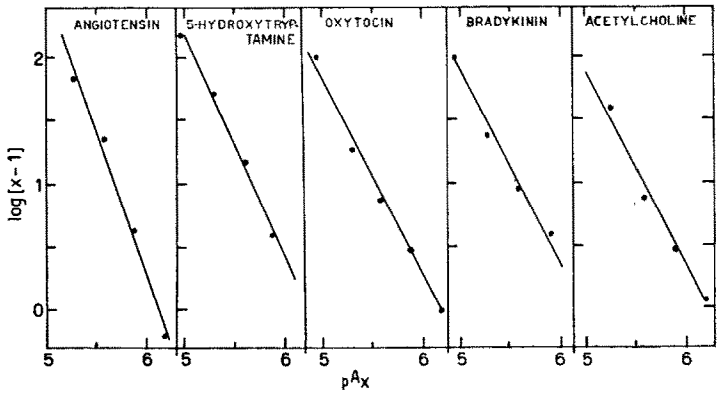

FIG. 5. The inhibition of angiotensin, 5-hydroxytryptamine, oxytocin, bradykinin, and acetylcholine by compound 46/108, plotted according to the equation: log $(x - 1)$ = log $K_B - n\mathrm{p}A_x$ (see text).

6·0 and 6·2. The slopes were also very similar, ranging between 1·75 for acetylcholine and 2·10 for angiotensin. The obtention of straight lines in these plots is more evidence that the inhibition is of the reversible competitive type.[8] However, according to the model used in the derivation of equation (1), the finding of slopes close to the value -2 would mean that two molecules of antagonist would compete with one of the agonist for one receptor site. This is not easy to conceive if it is considered that the receptor site in question would be common for agonists ranging from smaller to greater molecular sizes than that of the antagonist. However, we find it even more difficult to propose a plausible alternative model of drug–antagonist–receptor interaction that would account for the observation that the inhibition varies with the square of the antagonist concentration.

Figure 5 shows that compound 46/108 inhibits the uterus response to angiotensin, 5-hydroxytryptamine, oxytocin, bradykinin, and acetylcholine in a very similar way, with no significant differences in the slopes or the intercepts at the abscissae. This is good provisional evidence[6] that in the uterine muscle cell there is a common receptor site for these five drugs, and that compounds 48/80 and 46/108 inhibit the response to these drugs by competing for that site. In view of the relatively large differences in

the chemical constitution of the agonists studied, which however have a common feature in their positively charged nitrogen atoms at the physiological pH, we think it is very possible that the common receptor site in question is an anionic group. It is interesting that compound 48/80 also inhibits the binding of these drugs to heparin, suggesting that a heparin-like polysaccharide might be a part of the smooth-muscle cell receptor, as proposed by Bell.[1] Unfortunately, the inhomogeneity of the heparin preparations used in the binding studies does not permit an evaluation of the association constants for that binding, which would be interesting to compare with the K_B values found for the inhibition of the myotropic activity.

In conclusion, our results show that the condensation products of *p*-methoxyphenethyl-methylamine are competitive inhibitors of the binding of several cationic myotropic drugs to a common anionic site in the uterine muscle cell receptor complex. Whether this anionic group is located in an acid polysaccharide or in some other molecule in the cell membrane structure, however, is a question awaiting further investigation.

Acknowledgements—Compounds 48/80 and 46/108 were obtained through the courtesy of Dr. R. Baltzly, Wellcome Research Laboratories, Tuckahoe, N.Y., and Dr. A. M. Rotschild, Faculty of Medicine of Ribeirão Prêto, S.P., Brazil. The authors are also indebted to Dr. R. Schwyzer, CIBA Ltd., Basel, for the gift of synthetic angiotensin peptides.

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