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N-Chloroacetyl 5-methoxytryptamine (isamide): a selective antagonist of 5-hydroxytryptamine in the rat uterus

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Isamide, the *N*-chloroacetyl derivative of 5-methoxytryptamine, produced a dose-dependent competitive blockade of uterine contractions in vitro induced by 5-HT. The pA_2 value for the 5-HT-isamide interaction was 4·42. The blockade was short-lasting and reversible; after recovery, a dose-dependent increase in the uterine sensitivity to 5-HT was found. The blockade proved to be selective to the 5-HT receptor. The simultaneous application of 5-HT plus isamide partially prevented the 5-HT-induced auto blockade phenomenon. In addition, isamide did not affect the contractile responses of the uterus to oxytocin or bradykinin or the contractile effects of the rat vas deferens to adrenaline.

The development of selective antagonists is a major goal of pharmacology and therapeutics. Baker (1967) proposed some basic rules for the design of active site directed molecules that could combine high affinity and specificity of effect. We have applied some of these rules to the field of neuropharmacology and modified the structure of 5-hydroxytryptamine (5-HT) in an attempt to obtain drugs that are selective for the 5-HT receptor sites. Among the anti-5-HT drugs available for clinical use, none selectively blockades 5-HT responses. On the contrary, one of the major side effects of these drugs is the low specificity of blockade produced (Goodman & Gilman 1975). The present study is a preliminary report on a potentially useful haloacetyl derivative of 5-HT that proved to be selective for 5-HT uterine receptors. Further modification of the structure of such a derivative might result in drugs that could be useful in the understanding of the pharmacology of 5-HT neurons and diseases related to 5-HT metabolism.

MATERIAL AND METHODS

Drugs

5-Hydroxytryptamine creatine sulphate and (−)-adrenaline bitartrate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Bradykinin was a generous gift from Professor H. Croxatto and oxytocin was obtained commercially from Sandoz (Syntocinon). All salts were of analytical grade from E. Merck (Darmstadt, Germany). *N*-Chloroacetyl 5-methoxytryptamine was synthesized by mixing 1mM of 5-methoxytryptamine with 1mM of chloroacetyl-chloride. A solid was recrystallized from toluene that gave one spot on silica gel plates for thin layer chromatography ($R_F = 0{\cdot}69$ in chloroform–ethyl-acetate 1:1). The compound was identified by n.m.r. spectra and deuterium exchange. For all biological studies, isamide was dissolved in propylene glycol. Doses of all drugs are expressed as the final molar concentration of the free bases.

In vitro preparations

(a) *Rat uterus.* Uteri were dissected from Sprague-Dawley rats (180–240 g) in natural oestrus and maintained at 37 °C in a 10 ml organ bath containing Krebs solution as described by Roblero et al (1976). (b) *Rat vas deferens.* Vasa deferentia were obtained from Sprague-Dawley rats (180–240 g) and placed in a 30 ml organ bath maintained at 37 °C with Tyrode solution as reported by Miranda (1976).

Quantitation of responses

Isometric muscular contractions were registered using a force displacement transducer (FC.03) connected to a Grass polygraph. Tissues were initially given 2 g of tension and equilibrated with the bath solution for 1 h before drug addition. The tension finally achieved was about 1 g. Before deriving dose effect curves, the tissues were stabilized to the responses of a challenge dose of 5-HT ($5{\cdot}5 \times 10^{-7}$M) or to the other agonist used. Agonists were applied for 20–30 s at 4 min intervals.

Results were expressed as percentages of the maximal contractile response. Dose-effect curves were plotted as percentages of contraction vs log agonist concentrations, which allowed the calculation of the agonist's 50% effective dose (ED50). The

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antagonist pA_2 value was calculated from a Schild plot that was obtained plotting log (5-HT dose ratio-1) vs log of isamide concentration, as detailed by Arunlakshana & Schild (1959). When blockade was challenged by a dose of an agonist, the contractile response of the agonist before the application of isamide was considered as the 100% response.

Experimental procedures for studying the interaction of 5-HT with isamide

After the derivation of 5-HT dose-effect curves, uteri were exposed to varying concentrations of isamide for 15 min. Immediately following the incubation, or 60 min later, agonist dose-effect curves were obtained.

To investigate whether the blockade produced by isamide on the uterus was related to the active site of the 5-HT receptor, a protection experiment was performed. In this, 5-HT auto blockade was compared to that produced by 5-HT in the presence of isamide and that produced by isamide alone. To perform this experiment, uteri were incubated with a desensitizing dose of $5{\cdot}5 \times 10^{-5}$M 5-HT for 15 min. The uterus was then washed out and $5{\cdot}5 \times 10^{-7}$M 5-HT was applied every 4 min until the contractile response to the challenge dose recovered. In a paired series of experiments, uteri were incubated with $2{\cdot}04 \times 10^{-4}$ or $4{\cdot}08 \times 10^{-5}$M isamide plus $5{\cdot}5 \times 10^{-5}$M 5-HT for a 15 min period and challenged with $5{\cdot}5 \times 10^{-7}$M 5-HT at regular intervals.

To study the specificity of the blockade produced by isamide, uteri were challenged with either 0·5 mU oxytocin or $1{\cdot}02 \times 10^{-8}$M bradykinin after the application of 1·2 or $2{\cdot}0 \times 10^{-4}$M isamide. Rat vasa deferentia were stimulated with $4{\cdot}66 \times 10^{-6}$M adrenaline before/after isamide. Results compare the response to the challenge dose of the agonist as a ratio after/before application of isamide.

Statistics

The two-tail Student's *t*-test was used to compare results obtained before/after isamide. Significance was set at a *P* value <0·05. Linear regression and correlation coefficient analysis was used for the Schild plot, as described by Schefler (1969).

RESULTS

Isamide blocked 5-HT-induced uterine contractions. Increasing doses of isamide proportionally displaced the 5-HT dose-response curve to the right in a parallel fashion, achieving maximal response (Fig. 1). Analysis of the Schild plot revealed a straight line with a 5-HT isamide pA_2 value of 4·42. The slope of the curve calculated after fitting the points by the method of the least squares was 1·72. The correlation coefficient for this line was r = 0·87. The pA_{10}-PA_2 estimate was 0·57; a value that is only apparent considering the slope of the curve.

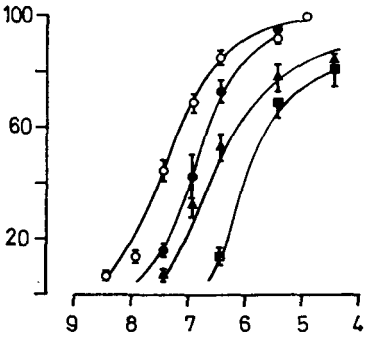

FIG. 1. Blocking effects of isamide on uterine 5-HT responses. Contractile responses (ordinate: % contraction) of the uterus to 5-HT were obtained 15 min after addition of isamide. (○) correspond to the control dose effect curve, before the application of the antagonist (n = 12). (●) refer to the responses obtained after application of $4{\cdot}08 \times 10^{-5}$ M isamide. (▲) and (■) refer to the 5-HT responses after application of $1{\cdot}20 \times 10^{-4}$ M and $2{\cdot}04 \times 10^{-4}$ M isamide respectively. Bars represent the s.e.m. of each value. Four uteri were used to obtain each of the 5-HT dose effect curves shown. Abscissa: −log (5-HT).

The blockade was completely reversible and of short duration. Fig. 2 shows the time course of the blockade produced by $2{\cdot}04 \times 10^{-4}$M isamide to challenges of $5{\cdot}5 \times 10^{-7}$M 5-HT. After the short-

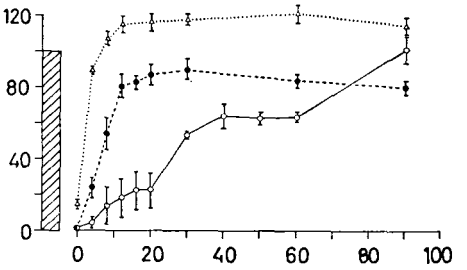

FIG. 2. Effect of isamide on the temporal course of the 5-HT auto blockade phenomenon. Recovery of 5-HT responses to challenges of $5{\cdot}5 \times 10^{-7}$ M 5-HT following a 15 min application of a densensitizing dose of $5{\cdot}5 \times 10^{-5}$ M 5-HT. Results are expressed as percentage of the 5-HT induced contractions before desensitization (ordinate). (○) represent the control values of the recovery of 5-HT responses alone. (●) refer to the recovery of the responses after the joint application of $4{\cdot}08 \times 10^{-5}$ M isamide plus $5{\cdot}5 \times 10^{-5}$ M 5-HT. (△) represent the effect of the addition of $2{\cdot}04 \times 10^{-4}$ M isamide alone. Symbols represent the mean ± s.e.m. of four different experiments in each case. Abscissa: time (min).

lasting blockade, there is a significant increase in the 5-HT responses. An hour after the addition of isamide, a dose-dependent potentiation of the effect of 5-HT was found. Table 1 shows a 2·27 and a 10·18 fold increase in 5-HT sensitivity following $4{\cdot}08 \times 10^{-5}$ and $2{\cdot}04 \times 10^{-4}$ M isamide respectively. The 5-HT dose effect curves were displaced to the left in a parallel fashion.

Table 1. Potentiation of the contractile effects of 5-hydroxytryptamine (5-HT) produced 1 h after the addition of isamide.

	5-HT ED50 $\times 10^{-8}$ M mean ± s.e.m.
Control (n = 8)	8·25 ± 2·02
$+4{\cdot}08 \times 10^{-5}$ M isamide (n = 4)	3·62 ± 1·66
$+2{\cdot}04 \times 10^{-4}$ M isamide (n = 4)	0·81 ± 0·18*

* $P < 0{\cdot}01$.

The blocking effect of isamide proved to be selective to the 5-HT receptor in the rat uterus. When isamide was applied with 5-HT, an almost complete protection of the 5-HT autoblockade (desensitization) was observed (Fig. 2). Furthermore, isamide did not antagonize the effect of oxytocin or bradykinin in the uterus under conditions of a dose-dependent antagonism of the 5-HT responses (Fig. 3). Neither did isamide antagonize the contractile effects of adrenaline in the rat vas deferens (Fig. 3). These results demonstrate a selectivity of blockade confined to 5-HT receptors in the rat uterine tissue.

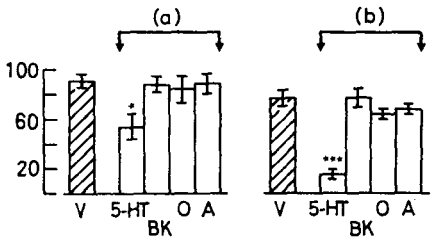

FIG. 3. Specificity of isamide's blockade on 5-HT responses. Columns represent the percentage of the contractile responses (ordinate) to challenge doses of agonists following the application of isamide (a) $1{\cdot}2 \times 10^{-4}$ M isamide, (b) $2{\cdot}04 \times 10^{-4}$ M isamide. Hatched columns represent the effect of the volume of propyleneglycol (vehicle = V) used in each addition of isamide. The challenge doses of the agonists were: $5{\cdot}5 \times 10^{-7}$ M 5-HT; bradykinin (BK) $1{\cdot}02 \times 10^{-8}$ M; oxytocin (O) 50 μU ml^{-1}; and $4{\cdot}66 \times 10^{-6}$ M adrenaline (A). (Adrenaline was studied in the rat vas deferens preparation, while all the other hormones were studied in the rat uterus.) A different uterine preparation was used per agonist studied; each experiment was repeated four times. Bars on the columns refer to the s.e.m. Note that only the responses to 5-HT were significantly reduced. * $P < 0{\cdot}05$; *** $P < 0{\cdot}001$.

DISCUSSION

Isamide produced a dose-dependent blockade of the 5-HT uterine responses with a pA_2 value of 4·42. The kinetics of the blockade can be interpreted as indicating a reversible, competitive 5-HT blockade. Due to technical difficulties caused by isamide's low affinity, the slope of the Schild plot might be apparent. Apart from 5-HT antagonism, other factors are presumably at play that can appreciably modify the real estimate of isamide's pA_2 value as has been pointed by MacKay (1978). Isamide could theoretically interfere with 5-HT uptake and/or metabolism, and perhaps isamide itself could be a substrate for uptake/metabolism. However, the present results suggest a competitive antagonism of isamide on the 5-HT uterine receptors. In addition, two experimental results support the conclusion that the blockade occurs at the active site of the 5-HT receptor, or at a site intimately connected with the receptor. Firstly, the selectivity of blockade indicates that the contractile machinery was not damaged by isamide. Furthermore, the muscular responses to oxytocin, adrenaline, or bradykinin were not modified by isamide, indicating an apparent selectivity to 5-HT receptors. Secondly, the results of the receptor protection experiment demonstrate that 5-HT and isamide compete for the same site, since isamide modified the kinetics of the 5-HT autoblockade (desensitization) phenomenon. In the presence of isamide, the recovery of the responses following a desensitizing dose of 5-HT was significantly faster, indicating that probably less 5-HT receptor sites were affected by 5-HT. The 5-HT autoblockade is thought to be a desensitization process mediated by the 5-HT receptor whereby after exposure to a high dose of 5-HT, the tissue becomes refractile to the effects of 5-HT. The molecular mechanism is apparently due to a decrease of active 5-HT receptor sites (Huidobro-Toro & Foree, unpublished results). In close analogy to 5-HT desensitization, Mukherjee & Lefkowitz (1976), Mukherjee et al (1976) and Lefkowitz et al (1976) presented evidence indicating that β-adrenoceptor desensitization involves inactivation and subsequent reactivation of existing receptor molecules (for a review see Wolfe et al 1977).

The antagonism of the 5-HT responses was followed by a state of increased sensitivity to 5-HT. The protection experiment indicated that the applica-

tion of 5-HT together with isamide not only prevented the antagonism of 5-HT, but prevented the subsequent increase in sensitivity to 5-HT. Furthermore, while the 5-HT blockade recovered rapidly, the potentiation of the 5-HT responses persisted for an hour following application of isamide. Hypothetically, the 5-HT supersensitivity could be considered to indicate blockade of silent receptors or of sites of 5-HT metabolism. These sites could correspond to extraneuronal, enzymatic or to transmitter uptake sites. The blockade of such zones would determine a time increase in the concentration of 5-HT available at the biophase, sensitizing the responses to 5-HT. Such receptors apparently have a different mechanism of interaction with isamide compared with the post synaptic muscle 5-HT receptor site. Thus, isamide or some derivatives could be of interest in further characterizing these two receptor sites.

Isamide, being a derivative of 5-methoxytryptamine was anticipated as having an intermediate affinity and selectivity for 5-HT receptors. The idea of synthesizing a chloroacetyl derivative of 5-HT was based on Baker's principle of an active site directed irreversible ligand–endo type (Baker 1970). The 5-methoxyindole portion of isamide could confer on the alkylating chain a moderate affinity and specificity of interaction for 5-HT receptors. Experimentally this was found to be so. Isamide has a low to intermediate affinity but a high degree of specificity for the 5-HT receptor. However, the affinity is rather low for a compound with a parent structure so closely related. It is known from structure activity studies that the methoxylation of the 5-hydroxyl group in the indole ring reduces the activity of the derivative as a 5-HT agonist (Erspamer 1954; Gyermek 1961; Huidobro-Toro & Foree, unpublished observations). This fact indicates that the 5-hydroxyl group must be free for 5-HT derivatives to exhibit the highest intrinsic activity for the 5-HT receptor. By analogy, the methoxylation of the hydroxyl group considerably reduces the affinity of isamide for the 5-HT receptor. It is expected that the *N*-iodoacetyl derivative of 5-HT might be a drug with increased affinity for 5-HT receptors, and a prolonged duration of action, as was found to be with chloroacetyl catechol and the *N*-iodoacetyl derivative of benzyl amine as α-adrenoceptor blocking agents (Huidobro-Toro & Carpi 1976; Huidobro-Toro et al, submitted for publication 1978). In contrast to the results obtained with isamide, Atlas & Levitzki (1976) and Atlas et al (1976) have recently reported on some bromoacetyl derivatives of β-adrenoceptor blockers that produced an apparent irreversible antagonism of the effect of catecholamines using in vitro preparations and Atlas & Levitzki (1976) noted that bromoacetylation markedly reduced the potency of several of the antagonist derivatives used.

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REFERENCES

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. 14: 48–58

Atlas, D., Levitzki, A. (1976). Biochem. Biophys. Res. Commun. 69: 397–403

Atlas, D., Steer, M. L., Levitzki, A. (1976) Proc. Natl. Acad. Sci. U.S.A. 73: 1921–1925

Baker, B. R. (1967) Design of active site-directed irreversible enzyme inhibitors. John Wiley and Sons, New York

Baker, B. R. (1970) Ann. Rev. Pharmacol. 10: 35–50

Erspamer, V. (1954) Pharmacol. Rev. 6: 425–487

Goodman, L. S., Gilman, A. (1975) The Pharmacological Basis of Therapeutics, Collier-MacMillan London 5th edn, p. 590

Gyermek, L. (1961) Pharmacol. Rev. 13: 399–439

Huidobro-Toro, J. P., Carpi, A. (1976) Arch. Int. Pharmacodyn. Ther. 222: 180–192

Lefkowitz, R. J., Mullikin, D., Caron, M. G. (1976) J. Biol. Chem. 251: 4686–4692

MacKay, D. (1978) J. Pharm. Pharmacol. 30: 312–313

Miranda, H. (1976) Arch. Int. Pharmacodyn. Ther. 221: 223–234

Mukherjee, C., Lefkowitz, R. J. (1976) Proc. Natl. Acad. Sci. U.S.A. 73: 1494–1498

Mukherjee, C., Caron, M. G., Lefkowitz, R. J. (1976) Endocrinology 99: 347–357

Roblero, J., Croxatto, H., Garcia, R., Corthorn, J., De Vito, E. (1976) Am. J. Physiol. 231: 1383–1389

Schefler, W. C. (1969). Statistics for the biological sciences. Addison-Wesley Publishing Co., Reading, Massachusetts

Wolfe, B. B., Harden, K., Molinoff, P. B. (1977) Annu. Rev. Pharmacol. 17: 575–604