Serotonin receptores in the isolated atria of Hamster

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ABSTRACT. Since LSD induces a positive chronotropic effect on isolated atria of hamster it is not suitable for characterizing specific serotonin receptors of auricles using cumulative dose-response curves. As isolated atria show neither the so called serotonin receptors M (blocked by morphine) nor nicotinic receptors, the positive chronotropic action of serotonin must relate to some other receptor systems. Serotonin has a transient negative chronotropic action of a cholinergic origin; however neither the direct interaction of indolamine with muscarinic receptors, nor the release of endogenous acetylcholine significantly affect the positive chronotropic action of the agonist on isolated atria.

RESUMEN. El efecto cronotropo positivo que el LSD induce en la aurícula aislada de hamster determina que esta droga no sea útil para caracterizar receptores serotonínicos específicos en las aurículas cuando se utilizan curvas acumulativas dosis-respuesta del agonista. Mostramos evidencias experimentales de que el efecto cronotropo positivo de la serotonina en la aurícula aislada de hamster no están implicados receptores M (bloqueables por morfina) ni tampoco receptores nicotínicos ni muscarínicos. Sin embargo, la acción cronotropa negativa inicial inducida por la serotonina en la aurícula es de origen colinérgico ya que es suprimida por atropina.

INTRODUCTION

In two previous papers we have demonstrated using various experimental designs that 5- hydroxytryptamine (5-HT; serotonin) has a dual action on the isolated atrium of hamster (IAH). One part of its positive chronotropic effect is directly mediated by interaction with specific serotonin receptors; the other is indirectly mediated by release of noradrenaline from adrenergic nerve terminals located in the heart[1].

We also found the stimulant action of serotonin on IAH to be unrelated to interaction with alpha adrenergic receptors[2]. To study further the receptors involved we have now used several drugs, either agonists of specific receptors or antagonists tested against the cumulative dose-response curve of 5-HT. They are: lysergic acid diethylamide (LSD) and morphine, antagonists of the so called D and M receptors for serotonin, respectively[3], atropine, antagonist of muscarinic cholinergic receptors; nicotine and hexamethonium, agonist and antagonist respectively of nicotinic receptors.

MATERIAL AND METHODS

Hamsters (120 to 160 g) of both sexes were killed by a blow on the head, hearts removed and atria dissected in Mac Ewen solution, composition in g/l NaCl, 7.6; KCl 0.42; $CaCl_2$, 0.24; $NaHCO_3$, 2.1; NaH_2PO_4; 0,143; glucose, 2; sucrose, 4.5. The atria were set up in an isolated organ bath of 40 ml capacity and attached to force displacement transducers connected to a Nihon Kohden polygraph to record spontaneous contractions. All experiments were carried out at 32°C with continuous bubbling of carbogen (95 % oxygen, 5 % CO_2). The initial tension of atria was adjusted to 0.5 g. For experiments, the preparations were left in the bath until spontaneous rate did not change by more than 5 beats/minute during one 10-minute interval; this took about 30 minutes. The bathing fluid was renewed every 10 minutes. Cumulative dose-response curves to serotonin were determined by a stepwise increase in concentration by a factor of three. Each dose of agonist was added as soon as the response to the previous administration had leveled off (at intervals of three minutes). Two cumulative dose-response curves to 5 HT were determined in each preparation (at intervals of 45 minutes). The antagonists were added 15 minutes prior to determining the dose-response curve to serotonin. They were kept in the organ bath throughout the experiments. Statistical calculations were performed according to conventional procedures[4]. In the legends of figures $\bar{x}_1$ constitutes the mean of basal atrial rate before treatment with antagonist, whereas $\bar{x}_2$ is the mean of atrial basal rate after pretreatment with it. These values are obtained prior to the beginning of the dose-response curves to 5-HT.

The following drugs were used: serotonin creatinin sulphate, lysergic acid diethylamide, morphine hydrochloride, nicotine bitartrate, hexamethonium bromide and atropine sulphate.

Throughout the text, all drug concentrations are expressed as molar concentrations.

RESULTS

Action of 5-HT on isolated atria of hamster

Sensitivity of IAH to serotonin is high. The pD_2 value for 5-HT was 6.2 (Fig. 1).

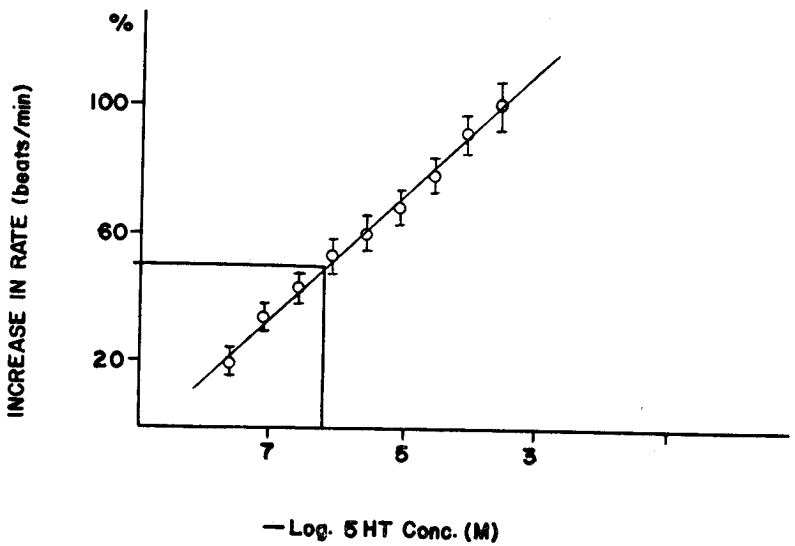

Fig. 1. pD_2 of HT in isolated spontaneously beating hamster atria X = 138 ± 5. Ordinate: increase in atrial rate in beats per minute. Abscissa: — log molar concentration of 5 HT. Shown are mean values ± S.E.M. of 19 experiments.

Action of LSD on the cumulative dose response curve to 5-HT in IAH

LSD (5×10^{-8} M) when added to the organ bath shifted the cumulative dose-response curve to the right, in parallel (Fig. 2).

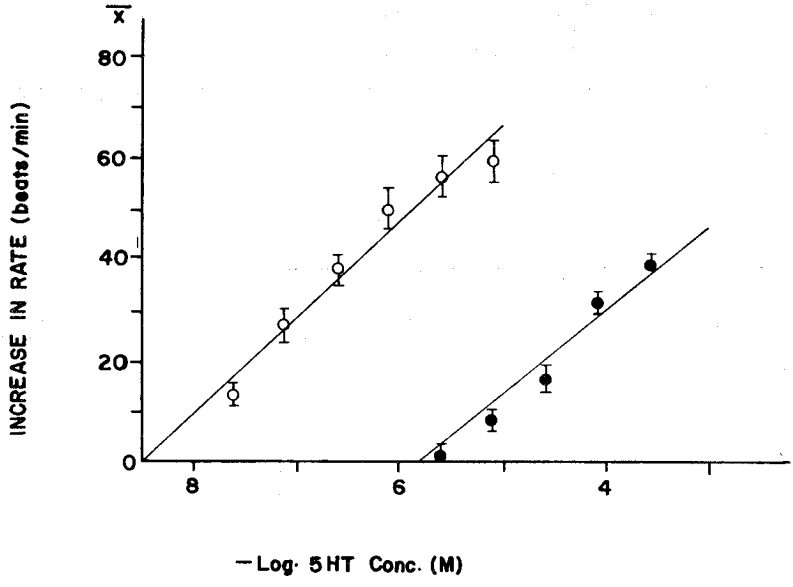

Fig. 2. Effect of LSD on dose-response curve to 5 HT in isolated spontaneously beating hamster atria. $X_1 = 129 \pm 9$ $X_2 = 143 \pm 9$. Ordinate: increase in atrial rate in beats per minute. Abscissa: — log molar concentration of 5-HT. LSD, 5 x 10^{-8}M was added to the organ bath 15 minutes prior to the beginning of the second dose response curve to 5HT. O—O, controls, ●—● LSD. Shown are mean values ± S.E.M. of 5 experiments.

However at this concentration and at 6.2 x 10^{-9} M, 1.2 x 10^{-8} and 10^{-7} M, LSD induced a positive chronotropic effect in IAH. The basal atrial rate being increased by 8 to 20 beats over controls.

The stimulant effect of LSD on atrial rate was concentration-dependent.

Action of morphine, atropine and hexamethonium on the cumulative dose response curve to 5 HT in IAH.

Morphine (1.85 x 10^{-4}M), atropine (1.78 x 10^{-6}M) and hexamethonium (5.5 x 10^{-5}M) failed to modify serotonin action significantly (Figs. 3, 4 and 5).

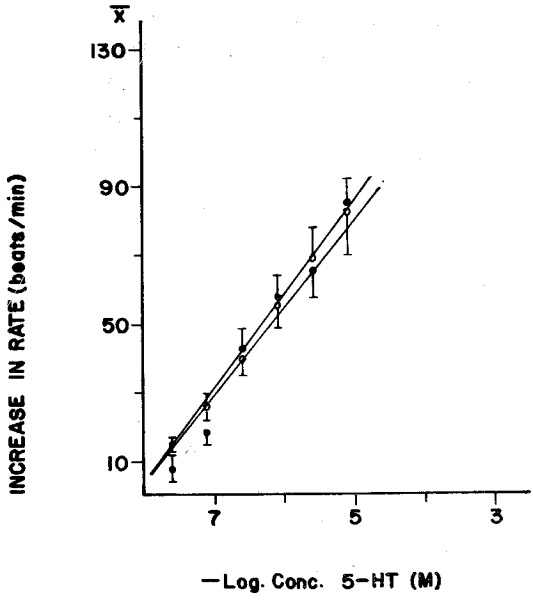

Fig. 3. Effect of morphine on dose-response curve to 5 HT in isolated spontaneously beating hamster atria. $X_1 = 139 \pm 8$ $X_2 = 139 \pm 12$. Ordinate: increase in atrial rate in beats per minute. Abscissa: —log molar concentration of 5 HT. Morphine, 1.85 $\times$ 10^{-4}M was added to the organ bath 15 minutes prior to the beginning of the second dose response curve to 5 HT. ○—○ controls, ●—● morphine. Shown are mean values ± S.E.M, of 6 experiments.

Action of nicotine on IAH

Concentrations of nicotine from 2 x 10^{-6} to 2 x 10^{-4}M added in a cumulative way lacked any IAH stimulant effect in atria either untreated, or pretreated with atropine.

DISCUSSION

Although LSD caused a parallel shift to the right of the cumulative serotonin dose-response curves (Fig. 2) we found it also to induce a positive chronotropic effect even at very low concentrations (2 to 32 ng/ml).

Since competitive antagonists must have affinity towards specific receptors, but no intrinsic activity, i.e. no ability to generate a stimulus[5]. We conclude that LSD is not a useful drug to study the serotonin receptors on the IAH by the cumulative dose-response curve method. In this case LSD might be considered as a partial agonist.

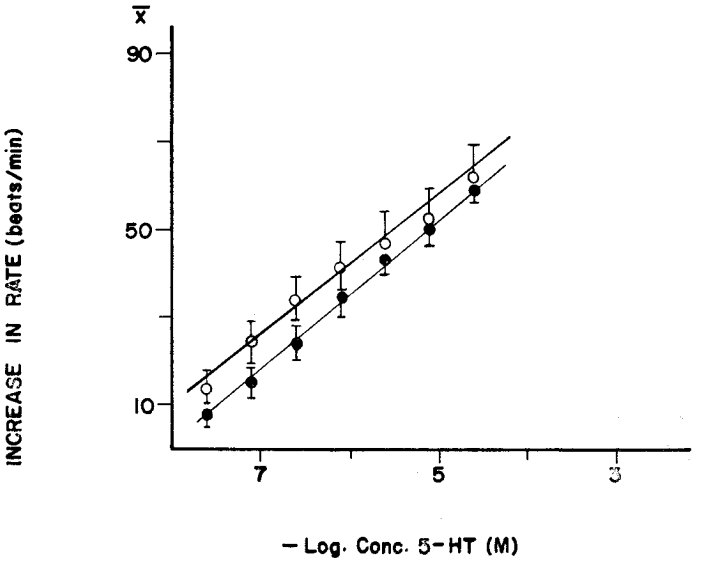

Fig. 4. Effect of atropine on dose-response curve to 5 HT in isolated spontaneously beating hamster atria. $X_1 = 160 \pm 9$ $X_2 = 163 \pm 10$. Ordinate: increase in atrial rate in beats per minute. Abscissa: — log molar concentration of 5 HT. Atropine, 1.78×10^{-6}M was added to the organ bath 15 minutes prior to the beginning of the dose-response curve to HT. ○—○ controls, ●—● atropine. Shown are mean values ± S.E.M. of 6 experiments.

Berridge and Prince[6] have reported a partial agonist action for LSD in the sallivary glands of an insect. There is considerable evidence on the interaction of LSD with specific serotonin receptors. Kier[7] using molecular orbital and quantum mechanic calculations concluded that 5-HT and LSD interact with the same receptor. There is a close structural similarity between 5-HT and LSD[8-10]. In particular, when the indole ring

of 5-HT or its derivatives is positioned over the corresponding region of LSD the positively charged nitrogen atom of both molecules coincides exactly[10].

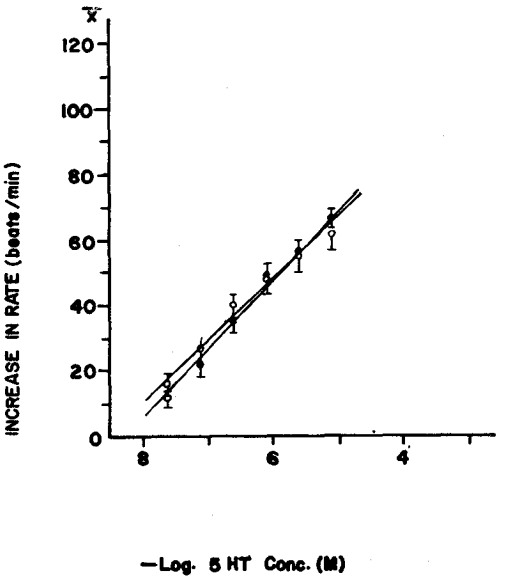

Fig. 5. Effect of hexamethonium on dose-response curve to 5 HT in isolated spontaneously beating hamster atria. $X_1 = 149 + 8$. $X_2 = 149 \pm 10$ Ordinate: increase in atrial rate in beats per minute. Abscissa: —log molar concentration of 5 HT. Hexamethonium, 5.5×10^{-5}M was added to the organ bath 15 minutes prior to the beginning of the dose-response curve to 5 HT. O—O controls, ●—● hexamethonium, Shown are mean values ± S.E.M. of 6 experiments.

Since the positively charged ethyl amine side chain appears to be the active site on the 5-HT molecule[11] there is reasonable justification for assuming that both molecules could activate the same receptor.

Since in our IAH test nicotine lacks stimulant effect and hexamethonium and morphine do not modify significantly the cumulative dose response curve to 5-HT it seems that nicotinic and serotonin receptors of type M do not exist in the IAH and the actions of serotonin are mediated by other means. Concerning the possible involvement of cardiac muscarinic receptors or endogenous acetylcholine release, in the chronotropic action of 5-HT in IAH, we found previously (data not shown) that 0.5 mcg/ml

of atropine inhibited completely the brief initial negative chronotropic effect induced by 5 mcg/ml of serotonin, but the later positive chronotropic effect was not modified. However, this cholinergic mechanism is not apparent when cumulative-dose response curves of 5-HT are used (Fig. 5). A possible explanation of these results may lie in the findings of Chittal et al.[12] that previous and continuous exposure of atria to 5-HT blocked the negative inotropic and chronotropic actions of acetylcholine added to the bath, 5-HT thus acting as a weak agonist on muscarinic receptors.

CONCLUSIONS

Serotonin has a transient negative chronotropic action of a cholinergic origin; however neither the direct interaction of 5-HT with muscarinic, M or nicotinic receptors, nor the release of endogenous acetylcholine significantly affect the positive chronotropic action of the agonist on isolated atria.

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