A POTENT COMPETITIVE INHIBITOR OF 5-HYDROXY- TRYPTAMINE : 3-(2'-BENZYLAMINOETHYL)- 5-METHOXYINDOL HYDROCHLORIDE

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During works on anti-5-hydroxytryptamine activity of tryptamine derivatives, 3-(2'-benzylaminoethyl)-5-methoxyindol hydrochloride (S-8) of which a chemical structure is shown in Chart 1 has been found to be a potent competitive inhibitor of 5-hydroxytryptamine (HT) action on the fundus of the isolated rat stomach, the isolated guinea pig uterus and the isolated guinea pig taenia caecum (taenia coli). It has been also found that S-8 depresses a rise of blood pressure of the rat caused by HT.

CHART 1. Chemical structure of 3-(2'-benzylaminoethyl)-5-methoxyindol hydrochloride (S-8). One gram dissolves about 100 ml water. m.p. = 194~196°C.

METHODS

A. Isolated organs

In order to obtain concentration-action curves, a cumulative technique was used (1).

1. The fundus strip preparation of the isolated stomach which was made according to Vane's method (2) was suspended in a 30 ml organ bath filled with Tyrode solution and kept at 32°C.

2. A piece of the uterus isolated from the estrous guinea pig was suspended in a 10 ml organ bath. Tyrode solution kept at 32°C was used as bath fluid.

3. A piece of the ileum from the male guinea pig was suspended in the 10 ml organ bath filled with Tyrode solution and kept at 32°C.

4. The vas deferens from the rat suspended in the 10 ml bath filled with Tyrode solution was used as a preparation to test effect of S-8 on the action of norcpinephrine. The fluid was kept at 32°C.

5. The tracheal muscle strip preparation from the male guinea pig was prepared according to Takagi, Takayanagi and Fujie's method (3). Tyrode solution kept at 38°C was used as bath fluid.

B. Blood pressure

Blood pressure of the rabbit or rat anesthetized with urethane (1.4 g/kg i.p.) was recorded through a mercury manometer connected to the right femoral artery. Intravenous injections of the drug solution were made into the femoral vein. The pithed rats were used in some experiments.

C. Membrane activity

The experiments were made on strips of the taenia caecum (taenia coli) from the guinea pig. A 40 mm *in situ* length of the ganglion free taenia caecum was dissected free from the underlying tissue and mounted in the sucrose gap apparatus according to the method described in the previous papers (4, 5). In this apparatus an arrangement was also made, using a mechano-electric transducer (RCA-5734) to record isometric contractions of the smooth muscle with electrical changes. All the results were presented as the mean of 10 experiments.

FIG. 1. The effects of S-8 on the concentration action curves of 5-hydroxytryptamine (HT) and ACh tested with the fundus of the isolated rat stomach.

Note that S-8 displaced the concentration action curve of HT to its higher concentrations in a parallel manner, indicating the competitive antagonism.

A : HT alone, B : HT with S-8 (3×10^{-7} g/ml), C : ACh alone, D : ACh with S-8 (3×10^{-7} g/ml).

RESULTS

1. *The fundal stomach preparation*

A concentration-action curve of HT was parallelly shifted towards its higher concentrations by the 15 to 30 minutes treatment of the preparation with S-8 in concentrations of 3×10^{-8} to 10^{-6} g/ml (Fig. 1). The pA_2 value of S-8 against HT which was calculated from the parallel shift of the curve of HT using van Rossum's table (6) was 7.94 ± 0.13 (mean $\pm$ standard error). However, lysergic acid diethylamide (LSD-25) in a concentration of 3×10^{-8} g/ml, which was classified as a typical anti-HT drug did not make a parallel shift of the concentration-action curve of HT but depressed the maximum height of the curve. S-8 in enough concentrations to inhibit the contraction caused by HT was without any effects on the contraction induced by acetylcholine (ACh). Moreover, S-8 in the higher concentrations (3×10^{-6} to 10^{-6} g/ml) produced contraction of the preparation, which was not inhibited by LSD-25 (3×10^{-8} g/ml).

2. *The guinea pig uterine preparation*

The concentration-action curve of HT was displaced towards its higher concentrations in a parallel manner by the 15 to 30 minutes treatment of the uterus with S-8 (10^{-7} to 3×10^{-6} g/ml), indicating a competitive antagonism between HT and S-8 (Fig. 2). The pA_2 value of S-8 against HT was 7.49 ± 0.14 (mean $\pm$ standard error). S-8 in concentrations of 10^{-7} to 10^{-6} g/ml was without any effects on the contraction caused by ACh. When

FIG. 2. The effect of S-8 on the concentration action curve of 5-hydroxytryptamine (HT) tested with the isolated guinea pig uterus.

Note that S-8 parallelly shifted the concentration action curve of HT towards its higher concentrations, indicating the competitive antagonism.

A : HT alone, B : HT with S-8 (3×10^{-7} g/ml), C : HT with S-8 (10^{-6} g/ml), D : HT with S-8 (3×10^{-6} g/ml).

3×10^{-6} g/ml of S-8 was used, the maximum height of the concentration-action curve of ACh was slightly depressed.

3. *The guinea pig ileum*

S-8 in concentrations of 10^{-7} to 10^{-6} g/ml depressed the maximum height of the curve of HT. The similar result was obtained in the experiments with LSD-25 (10^{-7} g/ml), as shown in Fig. 3. The contraction caused by ACh or histamine was not affected by S-8 in the concentrations of less than 10^{-6} g/ml, which were enough concentrations to inhibit the contraction produced by HT. Furthermore, response of the coaxially stimulated ileum from guinea pig was unaffected by S-8 in enough concentrations to inhibit the contraction of the same ileum induced by HT.

4. *The rat vas deferens*

S-8 in enough concentrations to antagonize the action of HT was without any effects on a concentration-action curve of norepinephrine. However, S-8 in the concentration of 10^{-6} g/ml caused some potentiation of the contraction by norepinephrine.

5. *The guinea pig tracheal muscle*

S-8 in concentration up to 10^{-6} g/ml was without any effects on the relaxation caused by epinephrine.

B. *Blood pressure*

In the anesthetized rabbits blood pressure was lowered after an intravenous administration of $100 \mu\text{g/kg}$ of HT. The pressure response by HT was greatly reduced by the pretreatment with S-8 (20 mg/kg). However, the pressure response by epinephrine was also reduced by S-8 in the same animals. In the anesthetized rats, blood pressure was raised by the intravenous injection of HT ($100 \mu\text{g/kg}$). The blood pressure response by HT was reversed by the pretreatment with S-8 (10 mg/kg), as shown in Fig. 4. In the pithed rats was also observed the rise of blood pressure by HT, which was greatly reduced by the pretreatment with S-8, as shown in Fig. 5.

C. *Membrane activity of the isolated taenia caecum (taenia coli)*

HT depolarized the membrane and made the spikes more frequent, at the same time as it induced a contraction of the taenia caecum. Responses of the preparation to HT were little or slightly affected by atropine (3×10^{-8} g/ml), morphine (10^{-5} g/ml) or tetro-

FIG. 3. The effect of S-8 and LSD-25 on the concentration action curve of 5-hydroxytryptamine (HT) tested with the isolated guinea pig ileum.

Note that S-8 and LSD-25 depress the maximum height of the curve of HT.

A : HT alone, B : HT with S-8 (10^{-6} g/ml), C : HT with LSD-25 (10^{-7} g/ml).

FIG. 4. The effect of S-8 on the pressure response of the urethane treated rat caused by 5-hydroxytryptamine (HT).

Note that the pressure response by HT was reversed by the intravenous injection of S-8.

Upper trace : blood pressure, lower trace : respiration.

() : minutes after the intravenous injection of S-8 (10 mg/kg).

FIG. 5. The effect of S-8 on the pressure response of the pithed rat caused by 5-hydroxytryptamine.

Note that S-8 depresses the pressure response caused by HT.

() : minutes after the intravenous injection of S-8 (10 mg/kg).

dotoxin (10^{-6} g/ml). These indicate that the site of action of HT is not in the nerve structures but on the smooth muscle in our preparations. After the 60 minute incubation of the taenia caecum with 10^{-7} g/ml of S-8, depolarization and the spikes caused by HT (3×10^{-7} g/ml) were abolished without any other effects on the membrane activity.

DISCUSSION

The above mentioned results indicate that S-8 selectively blocks the actions of HT. Furthermore, S-8 in its low concentrations of 10^{-8} to 10^{-7} g/ml greatly reduced the contractions of the rat fundal stomach and of the guinea pig uterus caused by HT but not the response of the guinea pig ileum to HT. This fact indicates that S-8 may have more affinity to the HT-receptor in the fundal stomach and uterus of the rat than to the HT-receptor in the cholinergic nerve structures of the guinea pig ileum.

In general, a competitive inhibitor abolishes the electrical and mechanical activities of the smooth muscle caused by its corresponding agonist without any effects on the spontaneous activities (5-7). As S-8 also abolished the electrical and mechanical activities of the ganglion free taenia caecum of the guinea pig caused by HT without any effects on the spontaneous activities, S-8 must be the competitive inhibitor of HT.

Since the contraction of the fundal stomach of the rat caused by the high concentrations of S-8 was unaffected by LSD-25, S-8 does not seem to have the HT-mimetic activity even in its high concentrations.

The rise of blood pressure of the anesthetized rats and pithed rats caused by HT was abolished by S-8 and the effects of HT on blood pressure of the anesthetized rats was reversed after the intravenous injection of S-8. It was rather difficult for S-8 to block the fall of blood pressure of the anesthetized rabbit caused by HT and enough dose of S-8 to inhibit the pressure response by HT also abolished the rise of blood pressure caused by epinephrine. It is given as a conclusion on blood pressure that S-8 selectively blocks the rise of blood pressure caused by HT.

SUMMARY

Anti-HT activity of 3-(2'-benzylaminoethyl)-5-methoxyindol hydrochloride (S-8) was tested, using the isolated fundus preparation of the rat stomach, the isolated guinea pig uterus, the isolated guinea pig ileum, the ganglion free taenia caecum (taenia coli) from the guinea pig and the experiments on blood pressure of the rat and of the rabbit. It is given as a conclusion that S-8 is a potent competitive inhibitor of HT, of which the pA_2 value is 7.94 on the rat fundal stomach and 7.49 on the guinea pig uterus.

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