Pharmacology Biochemistry & Behavior, Vol 38, pp 459–462 © Pergamon Press plc, 1991 Printed in the U S A 0091-3057/91 $3 00 + 00

RAPID COMMUNICATION

Peripheral 5-Carboxamidotryptamine (5-CT) Elicits Drinking by Stimulating 5-HT_1-Like Serotonergic Receptors in Rats

K. J. SIMANSKY

Department of Pharmacology, Medical College of Pennsylvania at Eastern Pennsylvania Psychiatric Institute 3200 Henry Avenue, Philadelphia, PA 19129

Received 26 September 1990

SIMANSKY, K J *Peripheral 5-carboxamidotryptamine (5-CT) elicits drinking by stimulating 5-HT_1-like serotonergic receptors in rats* PHARMACOL BIOCHEM BEHAV **38**(2) 459–462, 1991 —Subcutaneous administration of the prototypical 5-HT_1-like agonist, 5-carboxamidotryptamine (5-CT), increased 2-h water intake by nondeprived rats (ED_{50} = 0 04 μmol/kg) The 5-HT_1 agonists 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT, 0 04–0 32 μmol/kg) and RU 24969 (0 16 μmol/kg) did not produce drinking The dipsogenic effect of 5-CT (0.08 μmol/kg) was prevented by the 5-$HT_{1/2}$ antagonist, methysergide (ID_{50} = 4 μmol/kg), but not by 16 μmol/kg of the 5-HT_2 antagonist, ketanserin, the 5-$HT_{2/1C}$ antagonist, mianserin, or the 5-HT_3 antagonist, MDL 72222 5-CT also increased drinking and reduced food intake when food-deprived rats were given 2-h access to mash Methysergide (16 μmol/kg) inhibited both actions of 5-CT but an equimolar dose of the 5-HT_1/beta adrenergic antagonist, (−)-propranolol, blocked only the drinking The 5-$HT_{2/1C}$ antagonist, ritanserin (16 μmol/kg), altered neither ingestive action of 5-CT although, by itself, ritanserin increased mash intake The results suggest that activating a subtype of peripheral 5-HT_1-like receptor stimulates drinking in rats This receptor is unlike either the 5-HT_{1A} or the 5-HT_{1C} sites found in the brain Furthermore, the dipsogenic and anorectic actions of 5-CT occur independently

Serotonin 5-Hydroxytryptamine 5-Carboxamidotryptamine Serotonin analogs Serotonin agonists Serotonin antagonists Serotonin receptors 5-HT_1-like receptors Peripheral serotonergic mechanisms Drinking Feeding Anorexia

THE results of numerous studies have established that peripheral administration of serotonin (5-HT) increases water consumption by rats (8, 10, 11, 14, 15, 19). Serotonergic receptors in the periphery presumably mediate this action because it is questionable whether circulating 5-HT gains access to the central nervous system (18). However, the particular receptor involved in 5-HT-induced drinking has yet to be characterized.

At least three major classes of 5-HT receptor have been defined in the periphery· 5-HT_1-like receptors that are blocked by methysergide but not by ketanserin; 5-HT_2 receptors that are blocked by each of these antagonists, and 5-HT_3 receptors that are insensitive to these drugs but which are blocked by agents such as MDL 72222 and ICS 205-930 (2,16). Furthermore, pharmacological studies of serotonergic actions in various smooth muscles have suggested that subtypes of 5-HT_1-like receptors exist [e.g., (6)]. At least four subtypes of 5-HT_1 binding sites (1A–1D) have also been identified in the brains of mammals (9), although the extent to which they correspond to the peripheral subtypes remains controversial [e.g., (4,23)]. In previous studies, the nonselective 5-$HT_{1/2}$ antagonists, methysergide and metergoline, inhibited drinking produced by 5-HT (10, 11, 14, 15, 19), but the more selective 5-HT_2 antagonist, cinanserin, did not (10). These results implied that activating peripheral 5-HT_1 receptors elicited drinking by rats. This conclusion, however, is based upon limited data using antagonists and has yet to be challenged by experiments employing more selective agonists than 5-HT.

Based upon its actions in vivo and in vitro, the indole analog, 5-carboxamidotryptamine (5-CT), has been specified as the prototypical agonist at peripheral 5-HT_1-like receptors (2,16). The present study used this agent for probing further whether stimulating 5-HT_1 receptors would increase water intake by rats that were maintained with free access to food and water. Experiments were included for comparing the abilities of drugs with different relative 5-HT_1, 5-HT_2 and 5-HT_3 antagonist properties to inhibit the dipsogenic effects of 5-CT. On membranes from the brain, 5-CT binds avidly to the 1A, 1B and 1D subtypes, but displays only moderate affinity for the 1C subtype and very poor affinity for the 5-HT_2 class (9). Therefore, some of the antagonists were chosen because they differed in their relative actions among the 5-HT_1 sites. The actions of 5-CT in drinking were contrasted also with those of two nonindole 5-HT_1 agonists, 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT) and RU 24969 (9). Finally,

recent work from this laboratory established that 5-CT inhibits feeding by stimulating a methysergide-sensitive, ketanserin-insensitive receptor (22). In that study, some anorectic doses of 5-CT also increased drinking although the mechanism for this latter behavioral response was not explored. Accordingly, other experiments tested the interaction of 5-CT with certain antagonists on food and water consumption by rats given mash after mild deprivation. The results described below demonstrate that this indole increases water intake under a variety of conditions. Furthermore, this drinking response appears to be mediated, probably in the periphery, by 5-HT_1-like receptors and can be dissociated pharmacologically from the anorectic effect of the drug

METHOD

Male Sprague-Dawley rats (AAI, Inc.; Boyertown, PA) weighing 392–565 g at the time of the experiments were used in these studies. The rats were housed individually in suspended wire mesh cages (41 cm wide × 24 cm deep × 18 cm high) and maintained at an ambient temperature of 22 ± 1°C with a 12-h light photoperiod beginning at 06.00 h Water was available continuously from 100-ml graduated glass drinking tubes that were equipped with rubber stoppers and stainless steel spouts. In the experiments that determined only the effects of drugs on water intake, the rats had free access to standard pelleted laboratory chow (Prolab, Agway, Inc.; Syracuse, NY) except during the 2-h testing period. For studies comparing the actions of drugs on drinking and feeding, the rats were maintained with free access to sweetened milk in glass tubes from 14.00 h until 09.00 h the next day, deprived of food for 3 h and then given sweetened mash in clay dishes (9 cm diameter × 5 cm high) from 12.00 h until 14.00 h. The sweetened milk was prepared as described previously (22) by diluting one can (300 ml) of Carnation Sweetened Condensed Milk with 300 ml water and adding formaldehyde as a preservative to a final CH_2O concentration of 0 03% (w/v) The mash consisted of 400 g powdered rat chow mixed with 250 ml tap water and 50 ml of undiluted Carnation Sweetened Condensed Milk.

All testing was conducted in the rats' home cages. In the initial studies of drinking, the rats were injected subcutaneously (SC) between the scapulae with the appropriate serotonergic agonist or its vehicle and the volume of water consumed during the next 2 h was recorded. In subsequent experiments, antagonists were administered SC 30 min before 5-CT. When evaluating the ability of various antagonists to block the dipsogenic and anorectic actions of 5-CT, rats were injected first with antagonist or vehicle, 30 min later with 5-CT or its vehicle, mash and fresh water were provided 12 min later and the amounts of food and water consumed during the next 2 h were recorded. The combined weight of the mash and its clay dish was measured to the nearest 0.1 g using an Ohaus Port-O-Gram Balance. Mash intake was calculated as the difference between the weights at the start and end of the testing period and was corrected for spillage. Except as noted in the Results section, data were analyzed by Analysis of Variance followed by either two-tailed Dunnett's test or by Duncan's Multiple Range test. The threshold for significance was $p<0.05$.

For these studies, 5-CT (5-carboxamidotryptamine maleate, mol wt. = 319), 8-OH-DPAT [± 8-hydroxy-2-(di-N-propylamino)-tetralin hydrobromide, mol.wt. = 328], mianserin hydrochloride (mol.wt. = 301) and MDL 72222 (3-tropanyl-3,5-dichlorobenzoate, mol.wt. = 314) were purchased from Research Biochemicals, Inc (Natick, MA). (−)-Propranolol hydrochloride (mol wt. = 296) was purchased from Sigma (St. Louis, MO) Methysergide maleate (mol.wt. = 354, Sandoz Research Institute, East Hanover, NJ), ketanserin tartrate (mol.wt. = 545, Janssen Pharmaceutica Research Laboratories, Piscataway, NJ), ritanserin (mol wt = 478, Janssen) and RU 24969 [5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole hydrochloride, mol.wt. = 275; Roussel UCLAF, Roumainville, France] were donated generously by their manufacturers. A stock solution of 5-CT was prepared in distilled-deionized water that was bubbled with nitrogen, frozen at −70°C and aliquots were diluted with H_2O/N_2 just before injection. Ritanserin was moistened with two drops of Tween-80 and then suspended in water, MDL 72222 was dissolved in 0.05% (v/v) acetic acid; the other drugs were dissolved in water. Dilutions of the 5-CT stock, solutions of all other drugs and appropriate vehicles (VEH) were prepared on the day of each experiment with a constant volume of 2 ml/kg per injection.

RESULTS

5-CT increased water intake by nondeprived rats in a dose-related manner, $F(4,30) = 19.7$, $p<0$ 01, with a dose of 0.16 μmol/kg maximally stimulating drinking and 0.04 μmol/kg producing about half of this response (Fig. 1A) In contrast, rats did not drink more water after 0.04, 0.16 or 0.32 μmol/kg of 8-OH-DPAT (controls, 1 0 ± 0.4 ml; range of means after drug, 0 4–1.3 ml; n = 7 per group). In another experiment, rats drank 11.4 ± 1.8 ml after 0.16 μmol/kg 5-CT, but only 0.6 ± 0.3 ml after an equimolar dose of RU 24969.

Figure 1B shows that methysergide antagonized drinking produced by 0.08 μmol/kg 5-CT, $F(4,31) = 15.13$, $p<0.01$, with an approximate ID_{50} for methysergide of 4 μmol/kg and a dose of 16 μmol/kg eliminating the dipsogenic effect of the indole In contrast, pretreatment with 16 μmol/kg of either the 5-HT_2 antagonist ketanserin, the 5-$HT_{2/1C}$ antagonist mianserin or the 5-HT_3 antagonist MDL 72222 did not reduce significantly the water consumed after 0.08 μmol/kg of 5-CT. Specifically, in the experiment using ketanserin, controls (VEH + VEH) drank 0.9 ± 0 7 ml and 5-CT (VEH + 5-CT) increased this intake to 7.6 ± 0.6 ml. Ketanserin altered neither the baseline intake (KET + VEH, 0.1 ± 0.1 ml) nor the effect of 5-CT (KET + 5-CT, 6.6 ± 0.9 ml) In a separate experiment, 5-CT produced similar increases in water intake after pretreatment with vehicle (9.4 ± 0.9 ml) or mianserin [7.4 ± 1.4 ml; $t(13) = 1$ 17, NS, two-tailed Student's t-test]. Rats also drank comparable amounts of water after 5-CT regardless of the prior administration of the 5-HT_3 antagonist, MDL 72222 (VEH + VEH, 0 7 ± 0.3 ml; VEH + MDL, 1.0 ± 0 2 ml; VEH + 5-CT, 8.3 ± 1 0 ml; MDL + 5-CT, 10 7 ± 1 0 ml).

When administered to rats given access to a palatable mash diet after mild deprivation, 0 08 μmol/kg 5-CT not only increased 2-h water intake above that of controls but also concurrently reduced the amount of food eaten by 70–80 percent (Table 1). Pretreatment with 16 μmol/kg of methysergide antagonized both the anorectic and the dipsogenic actions of 5-CT, whereas the same dose of (−)-propranolol blocked only the increase in water intake. Ritanserin altered neither effect of 5-CT Controls drank more water in this latter experiment than in the study testing methysergide and propranolol. However, ritanserin did not change 5-CT-induced dipsogenesis in another test in which controls drank less (VEH + VEH, 1.2 ± 0.2 ml, VEH + 5-CT, 5 8 ± 1.4 ml, RIT + VEH, 0.8 ± 0 4 ml, RIT + 5-CT, 6.2 ± 1 6 ml) Notably, ritanserin increased food intake (by 33 percent) despite its failure to affect the anorectic action of 5-CT (Table 1).

DISCUSSION

These results clearly establish that systemically administered 5-CT stimulates rats to drink water In the present study, subcutaneously administered 5-CT increased the consumption of water by rats in the absence of food and also when the rats were con-

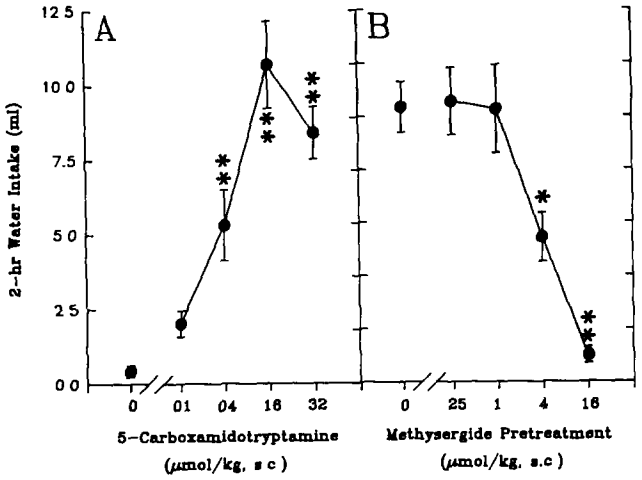

FIG 1 (A) Depicts the increased consumption of water elicited by subcutaneous administration of 5-carboxamidotryptamine (5-CT) in rats Data are expressed as means ± SE based upon 7 rats per group Water intake was measured for the 2-h period immediately following injection of 5-CT or its vehicle (0 dose). (B) Demonstrates the antagonism by methysergide of drinking produced by 5-CT. All rats were injected with 0 08 μmol/kg of 5-CT Thirty min earlier, rats were also given either one of the doses of methysergide (n = 7/group) or an injection of vehicle (0 dose for pretreatment, n = 8) Water intake was measured for the 2 h following injection of 5-CT In each panel, asterisks indicate a significant difference from the mean of the respective control group using two-tailed Dunnett's test after ANOVA *p<0 05, **p<0 01

TABLE 1

EFFECTS OF ANTAGONIST PRETREATMENT ON DRINKING AND ANOREXIA PRODUCED BY 5-CT

Pretreatment	Treatment	n	Water Intake (ml)	Mash Intake (g)
Vehicle	Vehicle	5	0 2 ± 0 2#	18 8 ± 4 4¶
	5-CT	5	5 0 ± 1 9†§	6 1 ± 1 2*‡
Methysergide	Vehicle	5	0 6 ± 0 2#	23 8 ± 3 8#
	5-CT	5	0 2 ± 0 2#	16 6 ± 0 8¶
(−)-Propranolol	Vehicle	5	0 4 ± 0 4#	16 7 ± 4 5¶
	5-CT	5	0 2 ± 0 2#	3 5 ± 1 4†§
Vehicle	Vehicle	7	4 1 ± 0 6#	18 6 ± 1 9#
	5-CT	7	10 6 ± 1 2†§	4 0 ± 1 0†§
Ritanserin	Vehicle	7	3 9 ± 0 3#	24 8 ± 2 2‡#
	5-CT	7	8 9 ± 1 0†§	4 6 ± 0 8†§

All values represent means ± SE for the amounts of water and food (mash) consumed during the 2-h period following the treatment with 5-CT (0 08 μmol/kg, SC) or its vehicle Pretreatments were given in a dose of 16 μmol/kg, SC, 30 min before 5-CT or vehicle Lines separate data from different experiments

*†Differs from corresponding Vehicle (control) group receiving the same pretreatment *p<0 05, †p<0 01

‡§Differs from Vehicle + Vehicle group ‡p<0 05, §p<0 01

¶#Differs from Vehicle + 5-CT group ¶p<0 05, #p<0 01

All comparisons by Duncan's Multiple Range Test after Analysis of Variance

currently eating moist food after mild deprivation. Previously, intraperitoneal injection of 5-CT elevated water intake in rats that were ingesting a liquid diet after overnight food deprivation (22). This indole, therefore, caused hyperdipsia under a variety of conditions of fluid and nutritional balance. Of particular importance, the dipsogenic action of 5-CT appears to occur independently of the stimuli associated with feeding.

The nonselective 5-HT$_{1/2}$ antagonist, methysergide, blocked 5-CT-induced drinking but the 5-HT$_2$ antagonist, ketanserin, and the 5-HT$_3$ antagonist, MDL 72222, did not. This pharmacological profile conforms exactly to that used for defining an action mediated by 5-HT$_1$-like receptors [e g., (2)]. In binding studies, methysergide displays high affinity for the 1C and 1D sites, moderate affinity for the 1A site and relatively poorer affinity for the 1B site (9). The 5-HT$_2$ antagonists, ritanserin and mianserin, also have very high affinity and potent blocking properties at 5-HT$_{1C}$ receptors (20), but they failed to inhibit the dipsogenic effect of 5-CT In prior work, the 5-HT$_{2/1C}$ agonist, α-methyl-5-HT (9,12), failed to increase water intake under conditions in which 5-CT did produce drinking (22). Thus, overall, the results suggest that 5-CT causes drinking by activating a 5-HT$_1$ receptor other than the 1C subtype. We have recently explored the effect of yohimbine, which has good affinity for 5-HT$_{1D}$ receptors (9) and blocks 5-HT actions at the rat fundus-type receptor (3), to inhibit 5-CT-induced drinking. Yohimbine increased both baseline water intake and serotonergic dipsogenesis (unpublished data), but it is not yet clear if these responses were due to serotonergic or, possibly, alpha$_2$ blocking properties of the drug.

In the present study, a dose of 8-OH-DPAT that was 8-fold larger than the ED$_{50}$ for 5-CT failed to increase drinking. A dose of RU 24969 equal to the maximally effective dose of 5-CT also had no effect on water intake. Each of these agents has high affinity for the 1A subtype; additionally, RU 24969 has high affinity for 1B sites and moderate affinity for 1D sites (9). The inability of 8-OH-DPAT to increase water intake extends previous results by others examining the hyperdipsic effects of 5-HT$_{1A}$ agonists in water-deprived rats (5). RU 24969 has not been shown to produce hyperdipsia and, in fact, reduced water consumption by thirsty rats (17) The lack of intrinsic activity of these nonindoles suggests that central 5-HT$_1$ receptors, especially of the 1A subtype, do not mediate drinking caused by systemic administration of serotonergic drugs The structure of 5-CT suggests that it penetrates poorly into the brain In the periphery, 5-HT$_1$-like receptors exist that are sensitive to 5-CT but not activated by either 8-OH-DPAT or RU 24969 [e g., (1,23)]. Testing additional doses of these agents and of other structurally dissimilar 5-HT$_1$ agonists will help to clarify the profile for drinking.

Propranolol blocked the dipsogenic but not the anorectic action of 5-CT These data establish that 5-CT reduces food intake independently of its ability to promote drinking. Single doses of propranolol have antagonized also the dipsogenic but not the anorectic effects of 5-HT (10,15). Besides its beta-adrenolytic actions, propranolol displays moderate affinity for the 1A, 1B and 1C subtypes of 5-HT receptors (9) Together, the present and other (10) data might favor an adrenolytic explanation for the action of propranolol in 5-HT-related drinking but dose-response studies comparing this drug with other adrenergic antagonists are required to address the issue (21)

An ancillary finding was that a very large dose of ritanserin increased food intake without altering the anorectic action of 5-CT. These data agree with a previously reported orexigenic action of ritanserin [(7); except, see also (13)]. Furthermore, they confirm our previous assertion that 5-CT acts at a 5-HT$_1$-like, rather than 5-HT$_2$, receptor to inhibit feeding (22). Moreover, these results argue against the involvement of a 5-HT$_{1C}$-type site in 5-CT-induced anorexia.

The major inference of this study is that selectively stimulating 5-HT$_1$-like receptors elicits drinking in rats The nature of the

receptor involved requires further analysis but it is probably located in the periphery and it is apparently unlike either the 5-HT_{1A} or the 5-HT_{1C} subtypes found in the brain. These conclusions derived from experiments using the indole derivative, 5-CT, as a more selective agonist than 5-HT itself for characterizing the pharmacological mechanism in question. 5-CT and related analogs should prove useful for exploring, in vivo, the specific physiological changes underlying 5-HT-induced drinking as well as other functional roles for 5-HT in the periphery

ACKNOWLEDGEMENTS

The author thanks James Jakubow, Frederick C Sisk and Kim Eberle-Wang for technical assistance with these experiments This research was supported by USPHS Grant MH 41987 from the National Institute of Mental Health

REFERENCES

1 Adler-Graschinsky, E , Elgoyhen, A B , Butta, N V Different receptor subtypes mediate the dual presynaptic effects of 5-hydroxytryptamine on peripheral sympathetic neurons J Auton Pharmacol 9 3–13, 1989
2 Bradley, P B , Engel, G , Feniuk, W , Fozard, J R , Humphrey, P P A , Middlemiss, D N , Mylecharane, E J , Richardson, B P , Saxena, P R Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine Neuropharmacology 25 563–576, 1986
3 Clineschmidt, B V , Reiss, D R , Pettibone, D J , Robinson, J L Characterization of 5-hydroxytryptamine receptors in rat stomach fundus J Pharmacol Exp Ther 235 696–708, 1985
4 Cohen, M 5-Hydroxytryptamine and non-vascular smooth muscle contraction and relaxation In Fozard, J R , ed The peripheral actions of 5-hydroxytryptamine New York Oxford University Press, 1989.201–219
5 Cooper, S J , Fryer, M J , Neill, J C Specific effect of putative 5-HT_{1A} agonists, 8-OH-DPAT and gepirone, to increase hypertonic saline consumption in the rat Evidence against a general hyperdipsic action Physiol Behav 43 533–537, 1988
6 Feniuk, W , Humphrey, P P A Mechanisms of 5-hydroxytryptamine-induced vasoconstriction In Fozard, J R , ed The peripheral actions of 5-hydroxytryptamine New York Oxford University Press, 1989 100–122
7 Fletcher, P J Increased food intake in satiated rats induced by the 5-HT antagonists methysergide, metergoline and ritanserin Psychopharmacology (Berlin) 96 237–242, 1988
8 Fletcher, P J , Burton, M J Effects of manipulations of peripheral serotonin on feeding and drinking in the rat Pharmacol Biochem Behav 20 835–840, 1984
9 Hoyer, D Functional correlates of serotonin 5-HT_1 recognition sites J Recept Res 8 59–81, 1988
10 Kikta, D C , Barney, C C , Threatte, R M , Fregley, M J , Rowland, N E , Greenleaf, J E On the mechanism of serotonin-induced dipsogenesis in the rat Pharmacol Biochem Behav 19 519–525, 1983
11 Kraly, F S , Simansky, K J , Coogan, L A , Trattner, M S Histamine and serotonin independently elicit drinking in the rat Physiol Behav 34 963–967, 1985
12 Leff, P , Martin, G R Peripheral 5-HT_2-like receptors Can they be classified with the available antagonists? Br J Pharmacol 88 585–593, 1986
13 Massi, M , Marini, S Effect of the 5-HT_2 antagonist ritanserin on food intake and on 5-HT-induced anorexia in the rat Pharmacol Biochem Behav 26 333–340, 1987
14 Meyer, D K , Abele, M , Hertting, G Influence of serotonin on water intake and the renin-angiotensin system in the rat Arch Int Pharmacodyn 212 130–140, 1974
15 Montgomery, A M J , Fletcher, P J , Burton, M J Behavioural and pharmacological investigations of 5-HT hypophagia and hyperdipsia Pharmacol Biochem Behav 25 23–28, 1986.
16 Mylecharane, E J The classification of 5-hydroxytryptamine receptors Clin Exp Pharmacol Physiol 16 517–522, 1989
17 Neill, J C , Cooper, S J Selective reduction by serotonergic agents of hypertonic saline consumption in rats evidence for possible 5-HT_{1C} receptor mediation Psychopharmacology (Berlin) 99 196–201, 1989
18 Oldendorf, W F Brain uptake of radiolabelled amino acids, amines and hexoses after arterial injection Am J Physiol 221 1629–1639, 1971
19 Rowland, N E , Caputo, F A , Fregly, M J Water intake induced in rats by serotonin and 5-hydroxytryptamine Different mechanisms? Brain Res Bull 18 501–508, 1987
20. Sanders-Bush, E , Breeding, M Putative selective 5-HT_2 antagonists block serotonin 5-HT-1c receptors in the choroid plexus J Pharmacol Exp Ther 247 169–173, 1988
21 Simansky, K J , Eberle-Wang, K Stimulating peripheral 5-HT_1-like serotonergic receptors increases water intake by a vagally-dependent mechanism in rats Soc Neurosci Abstr 16 294, 1990
22 Simansky, K J , Sisk, F C., Vaidya, A H , Eberle-Wang, K Peripherally administered α-methyl-5-hydroxytryptamine and 5-carboxamidotryptamine reduce food intake via different mechanisms in rats Behav Pharmacol 1 241–246, 1989–1990
23 Sumner, M J , Feniuk, W , Humphrey, P. P A Further characterization of the 5-HT receptor mediating vascular relaxation and elevation of cyclic AMP in porcine isolated vena cava Br J Pharmacol 97 292–300, 1989