PRESENCE OF ATYPICAL H_2-HISTAMINE RECEPTORS IN THE FROG SUBCLAVIAN VEIN

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UDC 612.134:612.731/.014.467:615.218.1

KEY WORDS: histamine receptors; tone of blood vessels; relaxation; vein; amphibians.

Effects of histamine and stimulators of histamine receptors (HR) of H_2-type (H_2-agonists) have been demonstrated in recent years on a number of smooth-muscle mammalian organs [5-8]. On the basis of these observations, Eyre and Chand [9] postulated the existence of atypical H_2-HR, or HR of the H_{2B}-subtype, by contrast with HR of the H_{2A}-subtype, which have the ordinary properties of H_2-HR. However, the question of the existence of HR of the H_{2B}-subtype has not yet been finally settled.

The drop of active mechanical tension of the frog subclavian vein (SV) induced by histamine likewise is blocked neither by the H_1-antagonist suprastin nor the H_2-antagonist IÉM-1044 [N-methyl-N'-4-(5-amino-1,2,4-triazolyl-3)-butylthiourea] [2], thus raising doubts about the participation of both H_1-HR and typical H_2-HR in the mechanism of relaxation [3].

The aim of this investigation was to test the hypothesis that this effect is realized through activation of H_{2B}-HR.

EXPERIMENTAL METHOD

The preparation (an isolated ring of SV of *Rana temporaria*), the technique, and the composition of the physiological saline were described previously [3, 4]. To observe the relaxing action, a conditioning contracture (CC) was induced beforehand with adrenalin in a dose of 10^{-6} or 10^{-5} M. The relaxing action of the substance was characterized by the value of pD_2, the negative logarithm to base 10 of EC_{50} ($-\log EC_{50}$), and by the index $|-\Delta T|_{max}/T$, where $|-\Delta T|_{max}$ denotes the maximal dimensionless relaxation of the test substance, T the value of CC before the beginning of relaxation (the index $|-\Delta T|_{max}/T$, where $|-\Delta T|_{max}/T$ is equivalent to the index Ri/R(t) in [3]). The value of EC_{50} was found from cumulative curves for the relationship between the concentration of the substance and the degree of relaxation produced by it $|-\Delta T|$. The value of $|-\Delta T|$ was determined as the difference between the tension of CC before creation of the first effective concentration of the substance in the bath and the tension established as a result of the action of the substance in that particular concentration. Histamine antagonists were injected 10-15 min before the beginning of CC, and histamine and the agonists began to be injected 30-40 min after the beginning of CC.

To identify HR, besides histamine and suprastin, the following substances also were used: 2-(2-aminoethyl)thiazole, a selective activator of H_1-HR (H_1-agonist), synthesized by the method in [1], the selective H_2-agonist dimaprit, and the selective H_2-antagonist cimetidine, which is more active than the IÉM-1044 used in [3]. The relaxing action of histamine, 2-(2-aminoethyl)thiazole, and dimaprit and modulation of the relaxing action of histamine by suprastin (suprastin hydrochloride, from Gedeon Richter, Hungary), in a concentration of 10^{-6} M, and of cimetidine, in a concentration of 10^{-4} M were investigated. The combination of agonists and antagonists used are given in Table 1. In three of the six experiments in which the relaxing action of histamine was observed in the absence of blocking agents, histamine base (from Fluka, Switzerland) was used, and in the other three experiments histamine hydrochloride (from histamine base) was used.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. G. Baranov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 593-595, November, 1985. Original article submitted July 12, 1984.

0007-4888/85/0011-1561$09.50

TABLE 1. Characteristics of Relaxing Action of Combinations of Histamine and Its Agonists and Antagonists on Rings of Frog Subclavian Vein

Series	Number of experiments	Agonist	Antagonist	pD_2 of agonist	$\lvert -\Delta T \rvert_{max} \mid T$
I	6	Histamine	—	3,6±0,2	0,9±0,1
II	4	"	Suprastin	3,9±0,2	0,8±0,1
III	3	"	Suprastin + cimetidine 10^{-4}	4,3±0,1	0,7±0,01
IV	3	"	Suprastin	3,4±0,3	0,7±0,01
V	3	2-(2-Aminoethyl)thiazole	"	3,4±0,3	0,9±0,1
VI	3	Dimaprit	"	4,5±0,1	1,4±0,3

Legend. In series IV CC was induced by adrenalin in a concentration of 10^{-5} M, in all other series in a dose of 10^{-6} M. In all cases suprastin was used in a concentration of 10^{-6} M.

EXPERIMENTAL RESULTS

In the absence of suprastin (experiments of series I) histamine, in low concentrations ($\leq 10^{-5}$ M) induced additional contraction, whereas in higher concentrations it induced relaxation. Histamine (series II, III, and IV), 2-(2-aminoethyl)thiazole (series V), and dimaprit (series VI) induced only relaxation in the presence of suprastin. The characteristics of the relaxing action of the combinations of agonists and antagonists are given in Table 1. It was found that dimaprit had the strongest relaxing action (as reflected in the value of pD_2 and of maximal dimensionless relaxation). Suprastin (series II) and a combination of suprastin with cimetidine (series III) caused an increase in sensitivity of the preparation to the relaxing action of histamine ($P = 0.25$ and $P = 0.01$ for series II and III respectively). The mean value of EC_{50} for the action of histamine on CC induced by adrenalin in a concentration of 10^{-5} M (series IV) was 4 times greater than that for CC induced in a concentration of 10^{-6} M (series II), but the difference was not significant ($P \varnothing = 0.2$). Values of pD and the index $\lvert -\Delta T \rvert_{max}/T$ for histamine base, in the action of blockers, were 3.5-0.2 (3 experiments) and 1.2-0.2 (3 experiments) respectively [3], and for histamine hydrochloride 3.7 ± 0.4 (3 experiments) and 0.6 ± 0.1 (3 experiments) respectively. Values for pooled data obtained with both histamine base and histamine hydrochloride are given in Table 1 (series I).

The absence of a blocking action of suprastin, confirmed by the present investigation (series II), indicates that H_1-HR are not involved in the relaxation of SV induced by histamine. Since this relaxation likewise was not blocked by either compound IEM-1044 [3] or by cimetidine (series III) it can be concluded that this effect also is realized without the participation of H_{2A}-HR.

These nonspecific (i.e., functioning in general without the participation of HR of any kind) mechanisms of the relaxing action of histamine and its antagonists as competition with adrenalin for α-adrenoreceptors (first mechanism) or for β-adrenoreceptors (second mechanism), or shifts of pH (third mechanism) must be ruled out. Adopting the first mechanism would require that the condition $\lvert -\Delta T \rvert_{max}/T \leq 1$ was observed for histamine and its agonists: in reality, for dimaprit $\lvert -\Delta T \rvert_{max}/T > 1$ (series VI). The first mechanism also is contradicted by the fact that the difference between the values of pD_2 for histamine in series II and IV was small (~1) and not significant. The second mechanism is unlikely, for the degree of relaxation induced by the β-agonist isoproterenol [3] is 1.5-2 times less than the degree of relaxation induced by histamine and its agonists. Unlike Hand and Buckner [10], we consider that the third nonspecific mechanism must also be ruled out, for the values of pD_2 for histamine base and histamine acid were virtually identical. Finally, dimaprit, which had the strongest relaxing action, does not induce measurable shifts in the pH of the medium even in a concentration of 10^{-3} M.

Meanwhile the relatively high value of pD_2 and the exceptionally strong relaxing power of the H_2-agonist dimaprit make it reasonable to deduce that relaxation initiated by histamine is realized through HR of the H_2-type. The fact that cimetidine cannot block this effect indicates that it is controlled by the HR of the H_{2B}-subtype also.

Despite the fact that values of pD_2 which were obtained for histamine and dimaprit, as agents initiating relaxation, were close to those given by other workers, who accepted the possible existence of atypical H_2-HR, the hypothesis relating to a nonspecific mechanism of relaxation remains a serious alternative to the assumption that relaxation is realized

through HR of the H_2B-subtype, primarily because of the high value of effective concentrations of the agonists studied ($EC_{50} = 3.3 \cdot 10^{-5} - 6 \cdot 10^{-4}$ M). Final proof of the presence or absence of atypical H_2-HR in the wall of the subclavian vein will be possible only after the elaboration of improved criteria and of methods of identification of H_2-HR subtypes than are currently available.

LITERATURE CITED

1. V. L. Gol'dfarb, L. L. Grechishkin, and L. K. Gavrovskaya, Author's Certificate No. 724512 (USSR) (1979).
2. L. L. Grechishkin, V. L. Gol'dfarb, and L. K. Gavrovskaya, Author's Certificate No. 596577 (USSR) (1977).
3. L. L. Protas, V. V. Sysoev, and G. R. Leont'eva, Byull. Éksp. Biol. Med., No. 1, 58 (1983).
4. V. V. Sysoev, in: Comparative Electrocardiology [in Russian], Leningrad (1981), pp. 35-37.
5. G. Bertaccini and L. Zappia, J. Pharm. Pharmacol., 33, 590 (1981).
6. N. Chand and P. Eyre, Agents Actions, 7, 183 (1977).
7. N. Chand, P. Eyre, and L. de Roth, Res. Comm. Chem. Path. Pharmacol., 23, 211 (1979).
8. P. Eyre and R. N. Besner, Res. Comm. Chem. Path. Pharmacol., 24 457, (1979).
9. P. Eyre and B. Chand, Agents Actions, 9, 1 (1979).
10. J. H. Hand and C. K. Buckner, Eur. J. Pharmacol., 70, 255 (1981).