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Frequency-dependent autoinhibition of histamine release from rat cortical slices: a possible role for H_3 receptor reserve

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Abstract—The inhibition of histamine release after depolarization of rat cerebral cortex slices by electrical stimulation and mediated by the postulated presynaptic autoreceptor (H_3) depends strongly on the conditions of stimulation. Using electrically stimulated slices of the cortex a rightwards shift of the concentration-response curve of histamine (an H_3 agonist) was observed on increasing the frequency of stimulation. The pA_2 value of the H_3 antagonist impromidine was, however, not altered at different stimulation frequencies; for a partial agonist only the maximal effect was influenced. These results indicate the existence of a receptor reserve at the H_3 autoreceptor.

During the last decade conclusive evidence has been gained for a definite function of histamine as a neurotransmitter in the central nervous system (cf. Schwartz et al 1982 for review). More recently it has been shown that, in rat brain, the release of histamine after tissue depolarization is under negative feedback control by a presynaptic autoreceptor (Arrang et al 1983). This so-called H_3-receptor has pharmacological characteristics different from H_1 and H_2 receptors. Histamine release and its blockade by exogenous histamine has been shown to be dependent on the conditions of stimulation, both after depolarization with K^+ and electrical stimulation (Arrang et al 1983; Van der Werf et al 1987a, b). In the present study this feature has been elaborated in more detail with the use of electrical stimulation applied to superfused brain slices of rats. Moreover, attention has been paid to the effects of impromidine (which antagonizes the response evoked by histamine) on the release of histamine under different conditions of stimulation. From the observations the existence of a frequency-dependent receptor reserve is postulated.

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Materials and methods

Chemicals and drugs. Histamine (HCl salt) was purchased from Aldrich (Belgium). Impromidine (HCl salt) was a gift from SK & F (UK). VUF8621 (a member of a series of side-chain derivatives of histamine: details to be published) was obtained from our laboratory stock. Dowex 50 WX4 (200–400 mesh) was obtained from Serva (Switzerland), L-[2,5-^{3}H]histidine (spec. act. ± 60 Ci mmol^{-1}) from Amersham International (UK). All other chemicals were from Merck (FRG) or Baker (The Netherlands).

Tissue preparation, superfusion and analysis of [^{3}H]histamine. The methods have been described by Van der Werf et al (1987a). Briefly the experimental setup was as follows. Male albino Wistar rats (190–200 g, TNO, The Netherlands) were decapitated, brains were removed and slices were prepared from the cerebral cortex. The slices were incubated in the presence of 150 μCi [^{3}H]histidine, washed to remove excess of [^{3}H]histidine and distributed among the 32 cells of a superfusion apparatus. Following equilibration, superfusion fractions were collected on chromatography columns. Depolarization of the slices was by electrical stimulation (biphasic 2 ms block pulses of 20 mA). In contrast to previous work, slices were depolarized only once. After superfusion, the slices were extracted by homogenization and centrifugation (Van der Werf et al 1987a).

In all fractions the amount of [^{3}H]histamine was determined by cation-exchange chromatography (DOWEX) and radioactivity was measured by liquid scintillation counting. The content of [^{3}H]histamine in each superfusion fraction was expressed relative to the total amount of [^{3}H]histamine present in the cell at the start of the collection of that fraction (= % release). Release observed after stimulation was always corrected for basal efflux by subtraction of the percent release measured in the preceding basal fraction. The p(EC50) values were calculated using ALLFIT (cf. Van der Werf et al 1987a). Statistical analysis was performed using Student's *t*-test.

Results

Frequency-dependent autoinhibition of histamine release. Increasing the frequency of stimulation led to a rightwards shift of the histamine concentration-response-curve (Fig. 1). Corresponding p(EC50) values were 8·1 ± 0·1 (1 Hz), 7·4 ± 0·1 (10 Hz), 7·0 ± 0·1 (20 Hz) and 6·6 ± 0·1 (33·3 Hz). Moreover, at the highest stimulation frequency tested (33·3 Hz), the [^{3}H]histamine release could not be completely inhibited (maximally about 80%) by exogenous histamine. At all other frequencies a complete inhibition could be so induced. When the slices were stimulated at a frequency of 10 Hz, a change in stimulation duration from 2 to 9 min did not affect the p(EC50) of histamine or its maximal blocking effect.

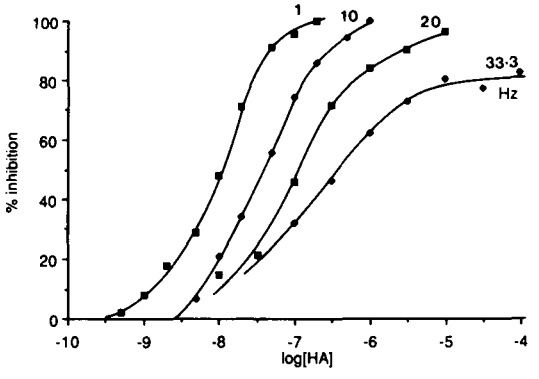

FIG. 1. Concentration-dependent inhibition of electrically-evoked [^{3}H]histamine release from superfused rat brain cortex slices by exogenously added histamine at different stimulation frequencies. Superfused rat brain cortex slices (previously loaded with [^{3}H]histamine) were electrically stimulated during 2 min, 90 min after onset of superfusion with various concentrations of histamine (HA) present in the superfusion medium. Inhibition data were derived from histamine release studies. Mean values of 3 to 6 determinations from 1 to 2 experiments are given, s.d. less than 10% of calculated values. Key: A 1Hz, B 10Hz, C 20Hz, D 33·3 Hz.

We have recently found a compound (VUF8621), which acts as a partial agonist at a frequency of 10 Hz, the release of histamine being inhibited by about 60%, with a p(EC50) value of 7·4 ± 0·3. When the stimulation frequency was reduced, the maximal inhibition induced increased to about 80% at 5 Hz, and at 1 Hz VUF8621 turned out to be a full agonist (100% inhibition). The p(EC50) was not significantly altered, 7·5 ± 0·2 at 5 Hz and 7·9 ± 0·2 at 1 Hz.

Effects of impromidine on histamine release at various frequencies. In previous studies we found that the release of histamine after stimulation is elevated when impromidine is present in the superfusion medium. The effect of impromidine on that release was also dependent on the frequency of stimulation (Table 1). At a stimulation frequency of 33·3 Hz, impromidine (10^{-6} M) did not influence [^{3}H]histamine release. At lower frequencies the release was elevated about 20% (10 and 20 Hz) and 60% (2 Hz) in the presence of 10^{-6} M impromidine.

Table 1. Effect of impromidine on [^{3}H]histamine release at various stimulation frequencies.

		Impromidine concn (M)		
Frequency	Control	10^{-7}	10^{-6}	10^{-5}
2 Hz[a]	8·8 ± 1·1	NM	14·3 ± 1·9[b] (+63%)	NM
10 Hz	21·3 ± 1·5	25·3 ± 2·4[b] (+19%)	25·9 ± 2·3[b] (+22%)	25·5 ± 1·4[b] (+20%)
20 Hz	28·7 ± 3·0	31·2 ± 1·7[ns]	35·5 ± 2·3[b] (+24%)	35·1 ± 2·0[b] (+22%)
33·3 Hz	37·8 ± 1·7	NM	39·5 ± 4·7[ns]	38·4 ± 3·1[ns]

Release % (± s.d.) are given, after stimulation with different frequencies for 2 min; values represent the means of 3 to 12 determinations from 1 to 3 experiments.
[a] in this case the duration of stimulation was 2·5 min.
[b] $P < 0·05$ as compared with corresponding control values.
[ns] not significantly different from the corresponding control value.
NM: not measured.

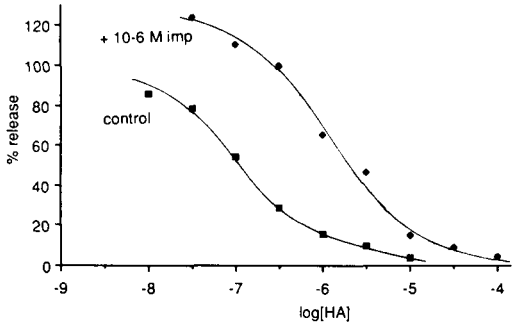

FIG. 2. Rightwards shift of the concentration-response-curve for histamine by 10^{-6} M impromidine at a frequency of 20 Hz. Superfused rat brain cortex slices (previously loaded with [^{3}H]histamine) were electrically stimulated during 2 min with a frequency of 20 Hz, 90 min after onset of superfusion with various concentrations of histamine (HA) present in the superfusion medium. Release data were calculated as indicated in the text. Mean values (s.d. less than 10%) of 3 to 6 determinations from 1 to 2 experiments are given. Impromidine clearly shows competitive antagonism. The p(EC50) value for histamine is lowered from 7·0 ± 0·2 in control to 5·9 ± 0·2 in the presence of 10^{-6} M impromidine. Key: ■ control, ● 10^{-6} M impromidine.

At a stimulation frequency of 10 Hz, a pA_2 value of 7·1 was found for impromidine (Van der Werf et al 1987a). At a stimulation of 20 Hz, impromidine showed competitive antagonism similar to that seen in Fig. 2. Calculation from the data from the shifted concentration-response-curve of histamine by impromidine at 20 Hz also resulted in a pA_2 value of 7·1.

Discussion

In previous studies we have shown that the autoreceptor (H_3) mediated inhibition of [^{3}H]histamine release depends on the frequency of stimulation (Van der Werf et al 1987a). In the present study we found that an increase in the stimulation frequency leads to a rightwards shift of the concentration-response-curve for histamine (Fig. 1). At 33·3 Hz the release of [^{3}H]histamine could no longer be completely inhibited by exogenous histamine; the maximal inhibition in this case was about 80%. At low stimulation frequencies (up to 20 Hz) the release of [^{3}H]histamine was enhanced by impromidine (Table 1). This increase is probably due to blockade of autoreceptors by impromidine, preventing inhibition of release by endogenous histamine (Van der Werf et al 1987a). In a previous study we

found that within the class of impromidine-like structures both H_2 agonists and H_2 antagonists showed H_3 antagonistic activity (Van der Werf et al 1987b). This indicates that H_2-mediated effects of impromidine on histamine release may be ruled out. At a stimulation frequency of 33·3 Hz, 10^{-6} and 10^{-5} M of impromidine had no significant effect on histamine release. Although at that frequency the release is greater than at lower frequencies, probably leading to higher synaptic concentrations, no significant inhibition of release by endogenous histamine was observed. At lower stimulation frequencies, however, lower levels of histamine lead to significant inhibition of histamine release, in accordance with the increase in its release after blockade of H_3-receptors by impromidine. These results are compatible with the data presented in Fig. 1; histamine is less potent at higher stimulation frequencies in inhibiting histamine release.

A frequency-dependent release of neurotransmitter has also been observed in other presynaptic receptor systems like those for noradrenaline (Langer et al 1975) and dopamine (Dubocovich & Hensler 1986).

Langer et al (1975) found that noradrenaline (NA, tested at $1{\cdot}8 \times 10^{-7}$ M) is less effective in inhibiting NA release in perfused cat spleen when higher stimulation frequencies are applied. They explained their results by assuming an enhanced entry of calcium ions at higher stimulation frequencies, leading to a less effective or desensitized negative feedback regulatory mechanism.

In their study, Dubocovich & Hensler (1986) observed that the potency of dopamine (DA) agonists at the DA autoreceptor from the rabbit retina is decreased at higher stimulation frequencies. Moreover, enhancement of DA release by D_2 receptor antagonists is increased at higher frequencies. They concluded that those effects are due to the fact that higher stimulation frequencies lead to higher synaptic concentrations of dopamine. As a consequence the agonists tested appear less potent at higher frequencies and the effect of antagonists is increased.

In our system, we did not find an increase in the effect of antagonists at higher stimulation frequencies, the effect was even impaired. The explanation given by Dubocovich & Hensler (1986) therefore does not seem appropriate in our case. Our hypothesis is that, at stimulation frequencies lower than about 20 Hz, in our system a receptor reserve exists. In such a system an increase in stimulation frequency will lead to a rightwards shift of the histamine concentration-response-curve, while the maximal effect (total inhibition of histamine release) is unaffected. This shift depends only on the stimulation frequency and not on the amount of histamine release, as longer duration of stimulation at 10 Hz did not affect the p(EC50) of histamine. At higher stimulation frequencies the maximal effect of histamine is decreased (from 100% inhibition at frequencies below 20 Hz to 80% at 33·3 Hz) which indicates that at these frequencies no receptor reserve remains.

Recently, a receptor reserve for the DA autoreceptor has been revealed after partial irreversible blockade of DA autoreceptors in-vivo (Meller et al 1986). The rightwards shift of concentration-response-curves for D_2 agonists as a function of the stimulation frequency, as observed by Dubocovich & Hensler (1986), can also be explained by the presence of spare receptors.

The assumption of a H_3 receptor reserve is further strengthened by the fact that no effect of the stimulation frequency on the pA_2 value of impromidine was observed. In general, for antagonists spare receptors have no effect on their blocking activity, as all receptors need to be occupied for a maximal blocking effect. For a partial agonist, only the maximal effect should be affected by a change in stimulation frequency, while the p(EC50) remains constant, according to this theory. Indeed this was found in our study for the partial agonist VUF8621. The maximal effect of this compound was enhanced when the stimulation frequency was lowered. At a frequency of 1 Hz VUF8621 became a full agonist (total inhibition of histamine release) while the p(EC50) was not significantly changed.

In conclusion the results of this study indicate that under certain stimulation conditions (stimulation frequencies below 20 Hz) a receptor reserve of the H_3 system exists. This finding is of importance for studies in which agonists are tested, and when data from different studies are being compared.

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