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Effects of Histamine, 5-Hydroxytryptamine and Their Antagonists on the Uterine Motility in the Rat

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Histamine, 5-Hydroxytryptamine 및 이들 拮抗物質이 흰쥐의 子宮筋 運動性에 미치는 영향

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抄錄 : 平滑筋으로 된 子宮筋은 自動性을 가지고 있어서 神經支配와 關係없이 筋自體로 運動을 하게 된다. 그러나 이러한 子宮筋의 形態的 및 機能的 正常狀態維持에는 estrogen의 作用이 不可缺한 要素로 되어 있으며 이 estrogen의 作用에 의하여 histamine의 子宮筋에 대한 作用이 受容體의 어떤 기전에 의한 것인지를 알기 위하여 本 硏究는 histamine과 5-hydroxytryptamine 및 이들 拮抗物質들의 子宮筋 運動性에 대한 收縮 및 弛緩作用을 調査하였다. 子宮筋의 運動性은 physiograph를 通해 子宮收縮의 頻度와 크기를 기록하여 아래와 같은 結論을 얻었다.

1. 5-hydroxytryptamine에 대한 phenoxybenzamine의 抑制作用은 phenoxybenzamine의 拮抗性의 結果이다.
2. histamine은 H_1-receptor를 통해서 흰쥐의 子宮平滑筋의 運動性은 增加한다.
3. 반면 histamine은 H_2-receptor를 통해서는 子宮平滑筋의 運動性을 弛緩시켰다.
4. 흰쥐의 子宮筋에서 H_2-receptor 遮斷劑가 H_1-receptor 遮斷劑의 作用보다 더욱 강하였다.

Introduction

The mechanism by which steroid hormones bring about implantation are not clear. There are suggestions of possible involvement of and mediation by histamine(Dey *et al.*, 1979; Kennedy, 1979; Oettel *et al.*, 1979; Saksena *et al.*, 1976).

Dispite considerable research, it is still not clear how estrogen initiates decidualization or implantation in the progesterone-primed uterus. On the basis of this and several other findings, it was proposed that the release of histamine, a vasoactive amine, by estrogen is responsible for induction of implantation (Dey *et al.*, 1979).

The process of implantation is restricted to a specific area in the uterus and requires the presence of a blastocyst. Since these process is an interaction between the uterus and the blastocyst, and since the blastocyst could be a source of histamine, it seems likely that the action of histamine would be linked between a blastocyst and the uterus(Johnson and Dey, 1980)

The liberation of histamine and subsquent edematous response are known to take place in the uterus within a few hours of estrogen stimulation(Szego, 1965). A similar temporal sequence of events would be

expected in the uterus after a nidatory surge of estrogen.

Histamine is generally thought to act as a neurotransmitter in the central nervous system. In the hypothalamus, histamine has both an excitatory and an inhibitory effect on neural firing activity.

The local inflammatory reaction observed at the site of blastocyst attachment may be bring about by an alteration in cyclic-AMP as a result of histamine action through H_1-receptor in the endometrium. Secondly, histamine acting via H_2-receptors on blastocyst cell membrane may stimulate cyclic-AMP formation which in turn may be involved in modulating the uterine immunological responses toward the embryo(conside red as an allograft) at the implantation site. Cyclic nucleotides have been shown to participate in implantation(Ash and Shild, 1966; Dey *et al.*, 1979).

Alternatively histamine participation may be mediated through the generation of prostaglandins, which have been implicated in the process of implantation (Kennedy, 1977; Oettel *et al.*, 1979).

Uterine receptivity at the time of implantation is controlled by a precise sequence of progesterone and estrogen action in the mouse and the rat. In the rat, the presence of progesterone for at least 48hrs followed by an estrogenic intervention is the basic hormonal sequence permitting implantation(Psychoyos, 1973).

Dey and his coworkers(1979; 1980 Dey *et al.*, a, b) have observed that the mammalian blastocysts have histamine forming capacity and that interference with this function interrupts embryo development as well as implantation.

Histamine stimulates the contraction of intestinal smooth muscle; this effect is mediated by H_1-receptors and can be suppressed by H_1-receptor antagonists such as mepyramine. Histamine also inhibits the contraction of the rat uterus and produces systemic vasodilatation in the dog; these effects are solely or predominantly mediated by H_2-receptors and blocked by H_2-receptor antagonists cimetidine and metiamide (Waldman *et al.*, 1977).

Specific H_1- and H_2-receptor antagonists in combination with histamine as well as specific H_1- and H_2-receptor agonists have been used to probe the nature of histamine receptors in smooth muscle of the gut. Longitudinal smooth muscle of the ileum in the guinea pig appears to contain only H_1-receptors which mediated contraction(Waldman *et al.*, 1977).

The amniotic level of 5-hydroxytryptamine, a biogenic amine, with potent vasomotor activity is higher during labor. The mechanism of 5-hydroxytryptamine action during the early stages of pregnancy has recieved little attentions. In mice and rats, single subcutaneous injection of 5-hydroxytryptamine have little or no effects before implantation, but marked antifertility effects subsquently, especially on the day of implantation(Marley, 1969, 1974).

Serotonin exerts a wide range of effects on the reproductive processes, including inhibition of ovulation(Wilson & McDonald, 1974), alteration of uterine blood flow(Clark *et al.*, 1980) and suppression of myometrial contractility.

Materials and Methods

Animal: The virgin female Sprague-Dawley rats weighing 150~200g were obtained from Laboratory Animal Breeding Center, Seoul National University. They were housed in a room maintained at 20~25°C with a 14h light phase followed by a 10h dark phase. They had access to food and water *ad libitum*. The rats were acclimated to their quaters for one week before the experiments were begun.

Uterine Motility: The rats recieved cumulative injection of E_2 before sacrifice. The E_2-primed rats were killed by cervical dislocation and the whole uteri were rapidly excised, cleaned and immediatly placed in Locke-Ringer's solution.

The uteri were cut into 2cm segments and longitudinal muscle strips were suspended in a 20ml organ bath of Locke-Ringer's solution at 37°C and gassed with pure oxygen. Muscle motilities were recorded isotonically using a isotonic myograph transducer (Narco Biosystem). The nutrient solution was freshly prepared before the experiment. The drugs used in this experiment were 5-HT, phenoxybenzamine, histamine hydrochloride, pyrilamine maleate and cimetidine. They were dissolved in isotonic saline containing 0.01M hydrochloride.

For each preparation, after a suitable equilibration

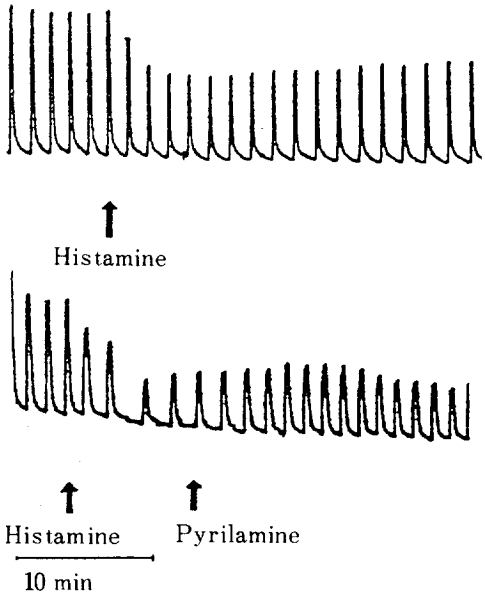

Fig. 1. The effect of pyrilamine(10^{-5}M) on the inhibitory response of the isolated rat uterus to histamine(5×10^{-5}M).

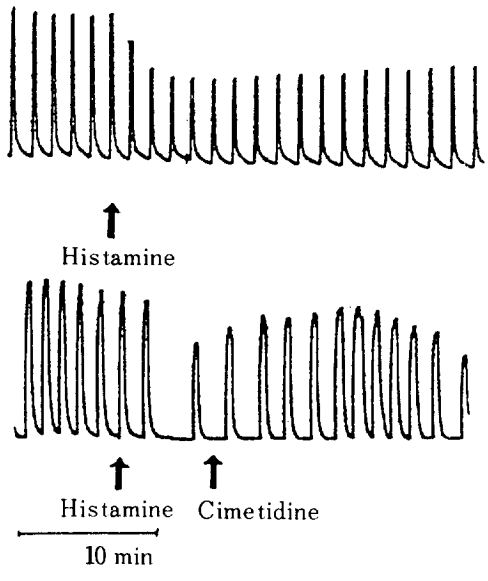

Fig. 2. The effect of cimetidine(10^{-5}M) on the inhibitory respone of the isolated rat uterus to histamine(5×10^{-5}M).

period, dose response curves to histamine(5×10^{-8}~10^{-4}M), ^{4}M), and 5-HT(5×10^{-8}~10^{-6}M final concentration) were obtained.

The antagonistic effects of pyrilamine(10^{-4} cimetidine(10^{-5}M) and phenoxybenzamine(10^{-5}M) on the responses of histamine(10^{-6}M) and 5-HT(10^{-5}M) to the longitudinal uterus strips were investigated.

Results

A dose response curve of the isolated rat uterus to histamine is shown in Fig. 1. Histamine at the concentration of 10^{-8}M did not inhibit normal contractility of the isolated uterus. Histamine(5×10^{-8}) caused a progressive inhibition in contractility. There was a nearly complete inhibition of this activity at histamine concentration frequency in 2min(Fig. 1).

The antagonistic effects of pyrilamine and cimetidine on the response of uterine smooth muscle produced by histamine are shown in Fig. 1. and 2. The inhibitory effect of histamine(10^{-4}M) on the uterine contractility was completely abolished by cimetidine (10^{-5}M) whereas pyrilamine(10^{-5}M) incompletely blocked the effect of histamine. A dose response curve of the isolated rat uterus to 5-HT is shown in Fig. 5.

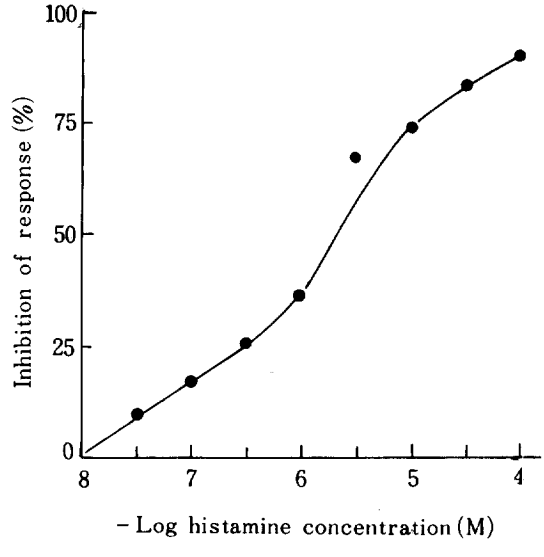

Fig. 3. Dose-response curve of the isolated rat uterus to histamine. The concentration of 5×10^{-8}M caused a progressive inhibition of the contractile activity. The nearly compelete inhibitory responses to histamine were at the concentration of 10^{-4} M.

No contractile response was seen in 5-HT(10^{-8}M), but the contractile response was increased with a dose of

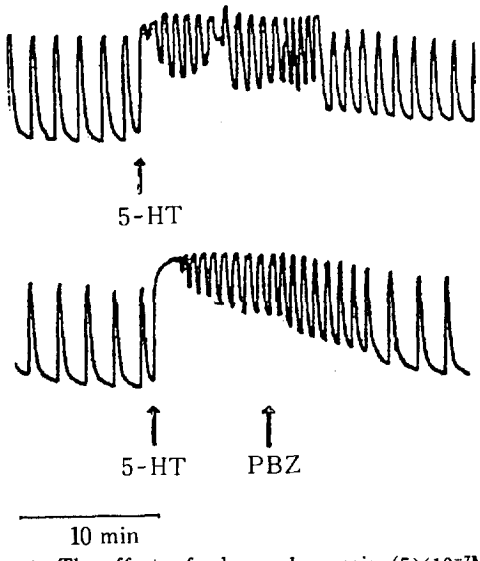

Fig. 4. The effect of phenoxybenzamine(5×10^{-7}M) on the contractile response of the isolated rat uteri to 5-hydroxytryptamine(5×10^{-6}M).
5HT : 5-Hydroxytryptamine
PBZ : phenoxybenzamine

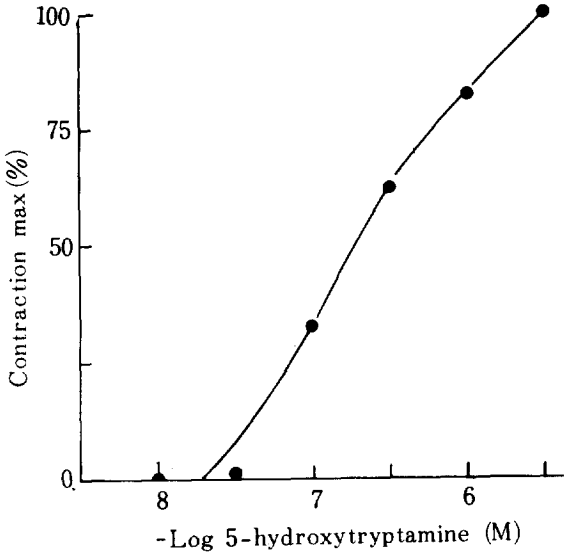

Fig. 5. The dose response curve of the isolated rat uteri to 5-hydroxytryptamine.

5×10^{-8}M The maxium contractile response was shown at a dose of 5×10^{-6}M. The results of Fig. 4 showed that phenoxybenzamine in the rat uterus. Phenoxybenzamine(5×10^{-7}M) blocked fully the contractile response produced by 5-HT(5×10^{-6}M).

Discussion

Histamine liberated from most uterine cells by estrogen stimulation may bring about a local inflammatory reaction by an alteration in cyclic-GMP as a result of histamine action through H receptors in the rabbit endometrium. Simultaneously, the liberated histamine may cause a proinflammatory response by mediation of prostaglandins generated as a result of the stimulation of phosphorylase-A activity. On the blastocyst side, histamine synthesized in the blastocyst may stimulate cyclic-AMP formation by acting through H_2-receptors and then cyclic-AMP may modulate the uterine immunological response toward the embryo.

The augmenting effect of histamine on the implantation rate could be partialy blocked by cimetidine H_2-receptor blocker, or pyrilamine, H_1-receptor antagonist. Interference with implantation by a combined treatment with cimetidine and pyrilamine was significantly enhanced but not completely(40%). The present studies have shown that H_2-receptor antagonist was more potent than the H_1-receptor antagonist on the smooth muscle of the isolated rat uterus, since the inhibitory response to histamine was completely abolished by the administration of cimetidine 10^{-5}M, but not pyrilamine 10^{-5}M. It is clearly demonstrated that H_2-receptors are distributed throughout the rat uterus. H_2-receptors of the uterus also mediated a muscle relaxant effect which was concentration dependant.

The results of the present experiments demonstrate the existance of H_2-receptors with a relaxant effect of histamine in the myometrium of the rat uterus. This evidence suggests the possibility that. the H_2-receptor may be involved in the motility of the uterus. The inhibitory response induced by histamine couldn't be blocked by pyrilamine, but could be blocked by cimetidine in the experiments for uterine motility. In the experiments for implantation, the augmenting effect of implantation produced by histamine was blocked by pyrilamine or cimetidine to the same degree. Thus the present studies indicate that a combination of H_1 and H_2 antagonist couldn't completely block implantation due to several factors:

i) H_1 and H_2 antagonists may stimulate rather than block the activity of histidine decarboxylase, a key enzyme for synthesis of histamine, in the blastocyst rather than block the activity(Hakanson *et al.*, 1977) and lately

ii) the antagonists may alter the gonadotropin or steroid hormons profiles by working via the pituitary or the ovary.

The result demonstrated that phenoxybenzamine in the uterine smooth muscle blocked the response to 5-HT while having little effects on the response of other contractile agent(Osman, 1975). Since exposure of the uterine tissue to phenoxybenzamine didn't affect the response of other contractile agent such as acetylcholine or oxytocin, it could be suggested that the response to 5-HT is acutally the result of drug antagonism and not due to general depression of the contractile process or metabolism.

It is already well known that histamine stimulates or inhibits the motility of smooth muscle by acting through H_1 or H_2-receptors. In the rat uterus, histamine induced relaxation by acting through H_2-recep tor and that such an effect of this amine was shown to be absolutely independant of the action of H_1-receptor.

Conclusions

This study has been carried out to investigate the augmenting or inhibiting effects of histamine, 5-hydroxytryptamine and their antagonists on the uterine motility.

The uterine motility was represented by the magnitude of impulse and the frequency of uterine contraction which was counted by the number of waves on the recording paper.

The results obtained were as follows :

1. The inhibitory effect of phenoxybenzamine on the response to 5-hydroxytryptamine is the result of drug antagonism.

2. Histamine stimulates or inhibits the motility of smooth muscle by through H_1 or H_2-receptor.

3. The uterine motility was increased through H_1-receptor.

4. Histamine induced relaxation by acting through H_2-receptor.

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