Effect of mepyramine, a histamine H₁-, and burimamide, a histamine H₂-receptor antagonist, on ovum implantation in the rat

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Summary. Systemic administration to rats of a combination of mepyramine, a histamine H₁-, and burimamide, a histamine H₂-receptor antagonist, over a period which included the time of implantation (late Day 5 to early Night 5), increased the number of blastocysts recovered from the uterus and reduced the number and intensity of Pontamine Sky Blue (PSB) sites obtained at autopsy on Night 5. Histological examination of the PSB sites taken on Night 5 from animals receiving the histamine antagonists revealed that the stromal oedema, which is characteristic of the attachment phase of pregnancy, had been inhibited.

Introduction

Oestrogen is required to initiate implantation in the rat (Krehbiel, 1941; Cochrane & Meyer, 1957). The association of oestrogen and of histamine, with hyperaemic and oedematous responses is well known and local application of histamine mimics the hyperaemic (Holden, 1939) and oedematous (Spaziani, 1963) responses seen after local or parenteral oestrogen treatment. The uterine histamine content has been reported to fall after oestrogen administration (Spaziani & Szego, 1958; Shelesnyak, 1959a; McKercher, Van Orden, Bhatnagar & Burke, 1973) and at the time of implantation (Shelesnyak, 1959b).

Shelesnyak (1952) proposed that histamine was responsible for the induction of decidualization but this theory did not gain general acceptance (for review see De Feo, 1967). The evidence for histamine as the inducer of decidualization was based largely upon studies using conventional antihistamines, such as diphenhydramine and promethazine, given locally into the uterine lumen (Shelesnyak, 1957), a procedure which has been shown to damage the uterine epithelium (Tachi, Tachi & Lindner, 1970). The conventional antihistamines do not antagonize all known actions of histamine, e.g. gastric acid secretion and the inhibition of induced contractions of the rat uterus. Ash & Schild (1966) defined the receptors at which the conventional antihistamines acted as histamine H₁-receptors. Black, Duncan, Durant, Ganellin & Parsons (1972) described a histamine antagonist, burimamide, which did not act at the H₁-receptors and defined the receptors at which such an antagonist acted as histamine H₂-receptors.

The present work was initiated to investigate the effect of systemic administration of mepyramine, a histamine H₁-, and burimamide, a histamine H₂-receptor antagonist, alone and in combination, on ovum implantation in the rat.

Materials and Methods

The I.C.I.-Wistar-derived rats used were from the Institute’s S.P.F. colony. The animals were maintained in a controlled environment, food and water being available ad libitum, from 21 days of age until they were used at 10–12 weeks of age. An artificial lighting cycle of 14 h fluorescent white light (21.00–11.00 h) and 10 h low-wattage red light was maintained at all times. Vaginal smears were taken from virgin rats for a minimum of 9 days and females displaying regular 4-day cycles were mated and randomly assigned to experimental groups. In this colony of rats, ovulation occurs from 1–5 h
after the midpoint of the dark period during which a pro-oestrous smear can be recorded and attachment is complete (10 or more Pontamine Sky Blue (PSB) sites per rat) 113 h after the time of 100% ovulation. The dark period during which mating and ovulation occurred was designated Night 0 of pregnancy, followed by Day 1, Night 1, Day 2 and so on.

Drugs were administered intraperitoneally at 09.00 h, 12.30 h and 16.00 h on Days/Nights 2, 3 and 4 of pregnancy, and at 09.00 h and 12.30 h on Day/Night 5. Burimamide (SK&F, Welwyn Garden City, U.K.), at a dose level of 63·6 mg kg⁻¹ (300 µmol kg⁻¹), and mepyramine (May & Baker, Dagenham, U.K.), at 5 mg kg⁻¹ (12·5 µmol kg⁻¹), were given as solutions in 0·9% (w/v) NaCl at an injection volume of 1 ml kg⁻¹. Animals were killed by carbon dioxide asphyxiation at 14.00 h (113 h after ovulation) or 16.00 h on Night 5, as indicated. In some experiments animals were given 1 ml 2% PSB (Searle, High Wycombe, U.K.) solution into a tail vein, 15–20 min before being killed.

The uteri were exposed and the number of PSB sites counted. In animals from which histological preparations were to be taken, the uteri were quickly removed and fixed (acetyl formol alcohol), dehydrated and cleared. Blue sites were cut from the cleared uteri and embedded in paraffin wax for sectioning (5–8 µm). Serial sections were stained with haematoxylin and eosin and each series examined. When blastocysts were to be flushed from the uterus, a fine ligature was tied across the oviduct and the horns were separated above the cervix. Each horn was flushed with 0·1 ml Medium 199 (Wellcome, Beckenham, U.K.) and the flushings were collected in transparent watchglasses and examined.

To compare treatment groups the data were divided into two categories: (i) rats with 0–5 flushed blastocysts or PSB sites, and (ii) rats with 6 or more flushed blastocysts or PSB sites, and the distributions were compared by using the χ² test or Fisher’s Exact Probability test.

Results

Treatment on Days/Nights 2–5 of pregnancy

Rats were treated with burimamide and/or mepyramine on Days/Nights 2, 3, 4 and 5 of pregnancy, at the time stated in ‘Methods’, and killed at 14.00 h on Night 5. When compared with saline-dosed controls, the combination of histamine antagonists resulted in a significant (P < 0·005) increase in the number of blastocysts recovered/rat on Night 5 (7·7 ± 0·6 (S.E.M.), N = 15 versus 2·6 ± 0·7, N = 14), and a significant (P < 0·025) decrease in the number of PSB sites/rat (8·0 ± 1·7, N = 9 versus 11·3 ± 0·7, N = 8). Neither antagonist alone had any significant effect on the number of blastocysts recovered on Night 5 (burimamide, 5·8 ± 1·2, N = 5; mepyramine, 4·0 ± 1·3, N = 5). It was noted in these and subsequent experiments that the PSB sites seen in animals given the combination of histamine antagonists were conspicuously paler than those seen in control rats.

Treatment on various Days/Nights of pregnancy

In a second series of experiments animals were given the combination of antagonists, at the doses stated previously, on various Days/Nights of pregnancy as shown in Table 1, and killed at 14.00 h on Night 5. There was a significantly (P < 0·001) greater number of blastocysts recovered/rat when treatment regimens including Day/Night 5 were compared with those excluding Day/Night 5.

Zona-encased blastocysts

A total of 622 blastocysts was recovered from 89 rats given the combination of histamine antagonists in any treatment regimen which included Day/Night 5, and 70 (11·2%) of these blastocysts were zona-encased. All of the 268 blastocysts recovered from 82 vehicle-dosed rats were zona-free.

Histology of attachment sites

Four PSB sites were taken from animals given the combination of histamine antagonists (Days/Nights 2–5, and Day/Night 5 only) and from vehicle-dosed control animals. The animals were killed at 16.00 h on Night 5.
Fig. 1. A Pontamine Sky Blue site from a rat given saline on Days/Nights 2–5 and killed on Night 5 of pregnancy. There is extensive oedema in the deep and subepithelial stroma. ×150.

Fig. 2. A Pontamine Sky Blue site from a rat treated with burimamide plus mepyramine on Days/Nights 2–5, and killed on Night 5 of pregnancy. Oedema is largely restricted to the antimesometrial deep stroma. ×150.
Table 1. The effect of burimamide (63.6 mg kg\(^{-1}\)) plus mepyramine (5 mg kg\(^{-1}\)) given at various times during early pregnancy on the number of blastocysts recovered from uteri on Night 5

<table>
<thead>
<tr>
<th>Day/Night of treatment</th>
<th>No. of rats</th>
<th>Mean ± S.E.M. blastocysts flushed/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 3, 4, 5</td>
<td>15</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>2, 3, 4</td>
<td>9</td>
<td>5.5 ± 1.7</td>
</tr>
<tr>
<td>3, 4, 5</td>
<td>7</td>
<td>6.3 ± 0.8</td>
</tr>
<tr>
<td>2, 3</td>
<td>8</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td>3, 4</td>
<td>7</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td>4, 5</td>
<td>26</td>
<td>7.1 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>5.3 ± 1.5</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>7.4 ± 0.7</td>
</tr>
</tbody>
</table>

All 4 of the sites from control animals contained a blastocyst in a well-defined implantation chamber (Pl. 1, Fig. 1). There was extensive deep and subepithelial stromal oedema and the eosinophilic stromal matrix was restricted to the mesometrial and deep lateral regions and areas around the glands. Only one of the 4 sites from animals given the antagonists on Days/Nights 2–5 contained a blastocyst (Pl. 1, Fig. 2). Stromal oedema was largely restricted to the deep antimesometrial region and the eosinophilic matrix was prominent in all areas. Three of the 4 sites from animals given the antagonists on Day/Night 5 contained blastocysts. The degree of stromal oedema present was intermediate between that illustrated in Pl. 1, Figs 1 and 2. The 4 sites from the antagonist-dosed animals that did not contain blastocysts showed no evidence of being empty implantation chambers. There was no extensive deep or subepithelial stromal oedema and uterine closure was retained in many of the sections. The occurrence of the PSB response in an area of the uterus which was not associated with a blastocyst or a decidual reaction has not been previously reported and no explanation of this finding can be offered at this time.

**Discussion**

Previous work in this laboratory has shown that, in this colony of rats, the period from 108–113 h after 100% ovulation (09.00–14.00 h, Day/Night 5) represents the time during which (a) zona loss occurs, (b) the number of blastocysts that can be flushed from the uterus falls to a minimum and (c) the Pontamine Sky Blue reaction develops. The present work has demonstrated that all of these events can be modified by the administration of a combination of mepyramine, a histamine H\(_1\)-, and burimamide, a histamine H\(_2\)-receptor antagonist, in a treatment schedule which includes Day/Night 5. The production of zona lytic factor (Surani, 1975), the increased adhesiveness of the luminal epithelium at the attachment phase of pregnancy (Nilsson, 1966, 1967), initiation of implantation (Krehbiel, 1941; Cochrane & Meyer, 1957) and the development of the PSB response (Psychoyos, 1960) are all oestrogen-dependent phenomena in the rat. The increase in vascular permeability at an attachment site (and the consequent local stromal oedema and PSB reaction) is initiated by an unknown stimulus from the blastocyst. During the present study this local response was greatly reduced, macroscopically and microscopically, by administration of the combination of histamine antagonists. The vascular reactions to histamine have been shown to involve H\(_1\)- and H\(_2\)-receptors in the cat, dog, rat and guinea-pig (Brimblecombe, Owen & Parsons, 1974; Black, Owen & Parsons, 1975). The implication of these results is that histamine may be involved in the mediation of some oestrogen-dependent phenomena and the local vascular response to the presence of a blastocyst. Inhibition of changes in vascular permeability (induced by oestrogen or the blastocyst) by histamine antagonists is consistent with the hypothesis of Schayer (1962) that histamine, formed as a result of the induction of histidine decarboxylase in or near the endothelium of small blood vessels, may act as a regulator of the microcirculation.
References


Received 5 October 1976