Abstract. The clinical improvement of Reynaud's disease during pregnancy has been attributed to the increased relaxin (RLX) level in blood. Therefore we investigated the effect of topical porcine RLX (NIH-RXN-P1) on the microcirculation of the mesoacem of Wistar male rats, under direct microscope observation as judged by two observers. The hormone was applied locally to avoid systemic interferences either alone or after norepinephrine (NE) and promethazine (PM). The drugs were coded and the results were recorded independently by two observers. Porcine RLX induced rapid dilatation of the veins of the rat mesoacem in a dose-related manner. The arteriolas and capillary flows were unchanged, while the venular flow was progressively reduced. The observed effects were sustained and disappeared with tissue wash-out. The RLX effects were reversed by addition of NE; conversely high doses of RLX were able to oppose the NE and PM vasoas. We conclude that the local administration of RLX influences the microcirculation, possibly through an action on the smooth muscle of the venulae. The effects seems antagonistic with those of NE and PM.

RLX has been known since 1926 (Hisaw 1926), but its physiological and pharmacological properties have not been fully elucidated mainly because of difficulties in its extraction and the low purity of the available preparations.

In recent years, the purification of RLX from the ovary of the pregnant pig, rat and shark (Schwabe 1983) and the recognition of the similarity of its structure with the insulin family (James et al. 1977; Schwabe et al. 1977) have aroused the interest of more researchers in the RLX field. It has been demonstrated that RLX is produced not only during pregnancy but also in non-pregnant females and in the male (Bryant-Greenwood 1982; Yki-Järvinen et al. 1983a,b) and is secreted not only by the corpus luteum but also by several other sources (Bigazzi et al. 1980, 1984).

Besides the well-known actions of relaxin in the pubic symphysis enlargement and the inhibition of uterine contractions, in recent years many different roles have been demonstrated or postulated for RLX both as a 'systemic' and as a 'local' hormone (Bryant-Greenwood 1982): it may be involved in important steps of the physio-pathology of reproduction and in the proliferation and differentiation of various components of the mammary glands (Bani et al. 1983, 1984). It is possible that RLX acts on the blood vessels, since we have recently observed an intense vasodilatation among the various effects produced by RLX on its target tissues. Casten et al. (1960) reported clinical ameliorations of peripheral arteriopathy following the administration of partially purified porcine RLX.

In this study we present evidence that RLX is capable of modifying the local microcirculation of the rat mesoacem when topically applied, influencing the venular vessels and the capillary, even in the presence of other vasoactive agents.

The local administration of relaxin induces changes in the microcirculation of the rat mesoacem

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Materials and Methods

The study was performed on male Wistar rats (100-200 g), fed with a standard balanced diet, following the method of Zweifach (1948), modified by Novelli et al. (1981). The animals were anaesthetised with pentobarbitone (30 mg/kg ip) and a small incision made in the lower abdomen, through which caecum and mesocaecum were exposed and perfused with a warmed, buffered, Ringer solution, pH 7.4, with 1% gelatine, to maintain the tissue temperature at 37 ± 1°C. The microvessels of the mesocaecum were observed by transillumination with a binocular microscope (100×).

The effect produced in this system by RLX was studied using $5 \times 10^{-6}, 1 \times 10^{-5}, 2 \times 10^{-5}$ porcine RLX (NIH-RXN-P1) dissolved in the warmed buffer and placed on the mesocaecum directly through a 50 µl micropipette, during the microscopical observation. Each dose was tested in 6 animals, at least.

In a second group of experiments we investigated whether the administration of porcine RLX (at the same doses as before) could antagonize the vasoconstrictory effect produced on the microcirculation by $1 \times 10^{-12}$ and $1 \times 10^{-7}$ NE and $6 \times 10^{-6}$ PM, dissolved in the same buffer at 37°C. The two drugs were added to the mesocaecum 30 sec after RLX.

Microcirculatory parameters

The functional status of the microcirculation before, during and after the local application of the drugs was evaluated according to the following parameters:

a) vasomotion (spontaneous opening and closing of capillaries);

b) venular flow (speed of blood flowing in the venulae);

c) arteriolar flow (speed of the blood flowing in the arterioles);

d) capillary flow (speed of the blood flowing in the functioning capillaries).

The effect on these parameters was evaluated sepa-

<table>
<thead>
<tr>
<th>Dose</th>
<th>Vasomotion</th>
<th>Venular flow</th>
<th>Arterioles flow</th>
<th>Capillary flow</th>
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<td>Controls (buffer alone)</td>
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<td></td>
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</tr>
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<tr>
<td>RLX + NE</td>
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<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>$0 + 1 \times 10^{-7}$</td>
<td>+3</td>
<td>+3</td>
<td>+3</td>
</tr>
<tr>
<td>RLX + NE*</td>
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<td>0</td>
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<tr>
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<tr>
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<td>+1</td>
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</tr>
</tbody>
</table>

* NE and PM were given to the mesocaecum 30 sec after RLX.
rately by two examiners who were not aware of the drug code, and the results expressed on an arbitrary scale from −4 to +4.

Results

The purified preparation of porcine RLX elicited a dose-dependent effect on the microcirculation of the rat mesoacema, producing a prompt dilatation of the veins and consequent reduction of the venular flow. The effect appeared immediately after the hormone deposition and remained unchanged, disappearing only with the wash-out of the tissue. Following RLX administration we did not observe any modification of the capillary flow and only sporadic and minor reduction in the arteriolar flow (Table 1).

RLX was able to antagonize the vasoconstrictory effect of NE and PM when applied before these drugs. In the presence of RLX, NE has no vasoconstrictory action; its effect was reversed by vasodilatation by porcine RLX on venules in a dose-effect relationship. Porcine RLX when applied at high doses is able to modulate the vasoconstrictor effect of PM.

Discussion

The functional status of the microcirculation is the result of many interrelationships acting on the various sites of the circulatory apparatus.

The model of topical application we have used for this study permits the direct observation of the effects of RLX and other drugs on the microvessels under conditions of maximal independence from the interferences of cardiac output, systemic constriction or dilatation, etc.

The dilatation of venulae and the consequent reduction of the venular flow produced by RLX in these experiments, are in accord with our previous observation (Del Mese et al. 1983). It is possible that the observed effects may derive from a reduction of the contractility of muscle cells of the venous walls produced by RLX, similar to RLX's effect on the myometrium, in which RLX induces a prompt inhibition of the spontaneous contractions and an active dilatation (Wiqvist & Paul 1958; Bigazzi et al. 1982). Our results may represent a local action of RLX rather than its systemic effect, deriving from a possible rapid passage of the hormone in the general circulation. In fact, even though we did not measure the blood level of RLX in the animals before and after the treatment, the quickness of the onset of the RLX effect and of its disappearance with the wash-out of the tissue depose for a local effect. This local and pharmacological effect of RLX does not exclude a possible systemic and physiological role on the vessels of this surprising hormone and our data may open the possibility of a new pharmacological tool in the treatment of vascular diseases.

In fact, we have observed that under the action of RLX, its target tissues undergo an intense vasodilatation and neoformation of blood vessels (Bani & Bigazzi 1984).

Furthermore, the effect of RLX on the microvascular system can be correlated with the unexplained clinical observation that Reynaud's phenomenon completely disappears during pregnancy (Casten & Boucek 1985) when RLX appears in the blood (Steinetz 1978) and with the striking amelioration of peripheral arteriopathy, observed by Casten et al. (1960) soon after treatment with porcine RLX, even if their preparation had a biological activity approximately 10% of that used in our experiments.

References


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