100, 1000 ng/ml of (±), (−) and (+) amphetamine in the presence of insulin, but obtained no significant increase in glucose uptake, except with 100 ng/ml of the (−) isomer, which gave an increase of 12.5%, significant at the 10% level.

We thank Prof. A.H. Beckett (Department of Pharmacy, Chelsea College of London University) for confirming the optical specificity of the isomers by g.l.c. separation and Servier Laboratories Ltd for supplying the isomers and for financial support.

References

CAMPBELL, D.B. (1971). Plasma concentrations of fenfluramine and its metabolite norfenfluramine after single and repeated oral administration. *Brit. J. Pharmac.*, **43**, 465P-466P.

FRAYN, K.N. & ADNITT, P.I. (1972). Effects of metformin on glucose uptake by isolated diaphragm from normal and diabetic rats. *Biochem. Pharmac.*, **21**, 3153-3162.

KIRBY, M.J. & TURNER, P. (1974). Effect of fenfluramine and norfenfluramine on glucose uptake by the isolated rat diaphragm. *Brit. J. Pharmac.* (in press).

Influence of prostaglandins and oestrus cycle on the spasmodic action of angiotensin II and oxytocin on rat uterus

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The uterine contractility is increased by oestrogen-treatment (Marshall, 1959) and depressed by progesterone (Kuriyama, 1961). On the other hand, the contribution of endogenous prostaglandins (PGs) to the oxytocin-induced contraction has been demonstrated (Vane & Williams, 1972).

The purpose of this work was:

1. To investigate the variations of the responses to angiotensin II, oxytocin and prostaglandin $F_{2\alpha}$, during the oestrus cycle.
2. To examine the possible role of prostaglandins as mediators of the spasmogenic action.
3. To look for some relation between the potentiating effect of sex hormones and the relative importance of the contribution made by endogenous PGs, angiotensin II- and oxytocin-induced contractions.

Uterine horns from Wistar rats were excised and equilibrated at 37°C in normal Ringer solution. Spontaneous and provoked contractions were measured isotonically.

The uterine contractility is *minimum in metaoestrus.* In this stage, the maximal responses are obtained with the respective concentrations of 5×10^{-7} M, 5×10^{-8} M and 10^{-5} M of angiotensin II, oxytocin and $PGF_{2\alpha}$, and the corresponding ED_{50} values are 10^{-8} M, 2.5×10^{-9} M and 10^{-6} M. Both the spontaneous activity and the sensitivity to agonists are *maximal in proestrus.* The maximal response and ED_{50} are observed at 10^{-7} M and 3×10^{-9} M for angiotensin, 5×10^{-9} M and 5×10^{-11} M for oxytocin, 5×10^{-6} M and less than 10^{-8} M for $PGF_{2\alpha}$ respectively.

For angiotensin II, the oestrus response does not differ from the proestrus and dioestrus responses. On the contrary, for oxytocin and $PGF_{2\alpha}$, the response in oestrus is intermediate between the metaoestrus and proestrus.

Indomethacin 5×10^{-6} M, which inhibits prostaglandin synthesis (Vane, 1971) shifts to the right the curves obtained with angiotensin and oxytocin in dioestrus and proestrus (respective ED_{50} 5×10^{-9} M and 2.5×10^{-10} M). It is inactive on these agonists in metaoestrus and on $PGF_{2\alpha}$ throughout the cycle.

Polyphloretin phosphate (PPP), a prostaglandin inhibitor administered at the concentration of 10 $\mu g\ ml^{-1}$ decreases the sensitivity of proestrus and dioestrus uteri to angiotensin (ED_{50} 10^{-8} M), oxytocin (ED_{50} 2.5×10^{-10} M) and $PGF_{2\alpha}$ (ED_{50} 10^{-7} M). A shift to the right of the metaoestrus curves is obtained at the concentration of 50 $\mu g\ ml^{-1}$.

On the contrary, a previous exposition to $PGF_{2\alpha}$ shifts the metaoestrus angiotensin dose-response curve towards the proestrus one.

These data demonstrate:

1. The physiological rôle of steroid-hormone impregnation as modulator of the uterine motility, for the oestrogen progesterone ratio is maximal in proestrus and minimal in metaoestrus (Hori, Ide & Miyake, 1968; Uchida, Kadowaki & Miyake, 1969).
2. A participation of endogenous PG in the contractile response to angiotensin II and oxytocin.
3. A good correlation between the importance of the contribution of endogenous PG to the contraction and the uterine hypersensitivity observed under oestrogen impregnation.

References

HORI, T., IDE, M. & MIYAKE, T. (1968). Ovarian oestrogen secretion during the oestrus cycle and under influence of exogenous gonadotropins in rats. *Endocr. Jap.*, **15**, 215-222.

KURIYAMA, H. (1961). The effects of progesterone and oxytocin on mouse myometrium. *J. Physiol.* (20 Nd), **159**, 26-29.

MARSHALL, J.M. (1959). Effects of oestrogen and progesterone on a single uterine muscle fiber in the rat. *Amer. J. Physiol.*, **197**, 935-942.

UCHIDA, K., KADOWAKI, M. & MIYAKE, T. (1969). Ovarian secretion of progesterone and 20α hydroxy pregn 4 en 3 one during rat oestrus cycle in chronological relation to pituitary release of luteinizing hormone. *Endocr. Jap.*, **16**, 227-237.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231**, 232-235.

VANE, J.R. & WILLIAMS, K.I. (1972). Prostaglandin production contributes to the contractions of the rat isolated uterus. *Br. J. Pharmac.*, **45**, 146P.

Effect of histamine on acetylcholine-evoked contractile responses of chronically denervated cat skeletal muscle

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In a previous study (Block & Reit, 1973) we found that histamine injected i.a. toward innervated skeletal muscles potentiated their contractile responses to acetylcholine injected shortly afterward *via* the same route. Potentiation also occurred when the acetylcholine was injected i.a. shortly after i.a. bradykinin or during exercise or post-occlusion hyperaemia, but when injected shortly after angiotensin, inhibition occurred. Therefore, we concluded that histamine exerted its potentiating effect by causing vasodilatation within the muscles, thus allowing more of the acetylcholine to reach more of its skeletal muscle receptors in a shorter time. We have now extended our studies of this vasomodulatory relationship to chronically denervated skeletal muscle.

Cats were anaesthetized with pentobarbitone sodium (35 mg/kg i.p.) and 2 cm of one peroneal nerve were removed aseptically. Fourteen days later, the cats were again anaesthetized and then rendered spinal. The denervated anterior tibialis muscles were prepared as previously described for recording their contractile responses, and the sural arteries were cannulated centrally so that i.a. injections could be made close to the muscles (Block & Reit, 1973). Drugs were dissolved in 0.9% NaCl solution and injected in volumes of 0.1 ml or less.

In the normally-innervated muscles, histamine (0.1 μg/kg) injected 15 s before graded doses of acetylcholine (3-100 μg/kg) caused a shift to the left of the acetylcholine dose-response curve of 1.5 log units. In the chronically denervated muscles, the control acetylcholine dose-response curve had become shifted predictably 3 log units to the left. But neither histamine (0.001-0.1 μg/kg) nor bradykin (0.01-0.1 μg/kg) shifted it any further, even though angiotensin (0.001-0.1 μg/kg) was still able to inhibit the supersensitive contractile responses to acetylcholine. Since, as Hudlicka (1967) has reported, the vascular bed of chronically denervated skeletal muscle is widely dilated compared to that of innervated control muscles, the vasodilator substances probably did not potentiate the acetylcholine-evoked responses of chronically denervated muscle because they could not increase significantly the already large nutritive blood flow within those muscles. Therefore, our results raise the question whether denervation supersensitivity to acetylcholine *in vivo* may be due not only to an increased surface area of cholinoceptivity on the individual muscle fibers but also in part to increased nutritive blood flow within the muscle mass. (Supported in part by PHS Grant R01-08259-04).

References

BLOCK, A.J. & REIT, E. (1973). The influence of histamine and other vaso-active substances on responses of cat skeletal muscle to acetylcholine. *Br. J. Pharmac.*, **49**, 74-85.

HUDLICKA, O. (1967). Blood flow and oxygen consumption in de-efferented muscles. *Circ. Res.*, **20**, 570-577.