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EFFECT OF TEMPERATURE ON CHANGES, INDUCED BY OESTRUS, in α- AND β-ADRENOCEPTOR ACTIVITIES OF THE RAT UTERUS

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The α- and β-adrenoceptor activities of the isolated rat uterus during the oestrous cycle were influenced greatly by temperature changes. During dioestrus, lowering the bath temperature to 25° C from 40° C increased two-fold the inhibition produced by (−)-adrenaline, (−)-noradrenaline, (−)-phenylephrine and (−)-isoprenaline. During oestrus adrenaline, noradrenaline and phenylephrine produced biphasic responses of contraction followed by inhibition at 40° C and isoprenaline produced only inhibition. At 25° C the contractile α-responses either were abolished (phenylephrine) or greatly reduced (adrenaline and noradrenaline), while the inhibitory β-effects of all four amines were increased.

It has been generally accepted since the time of Ahlquist (1948) that the uterus of the rat contains β-adrenoceptors. However, Mann (1949) found that the uterus of the rat in oestrus contracted to noradrenaline and that there was a biphasic response to adrenaline. Butterworth & Randall (1970) showed that these effects are due to the presence of both α- and β-adrenoceptors throughout the oestrous cycle. Some workers (e.g. Rudzik & Miller, 1962; Levy & Tozzi, 1963; Tothill, 1967) have disputed the presence of excitatory α-adrenoceptors within the normal oestrous cycle. There is now evidence that endogenous prostaglandins may be involved in motor responses of the uterus (Tothill, Rathbone & Willman, 1971; Aiken, 1972; Vane & Williams, 1973).

This paper describes a study of the effect of temperature on the relative α- and β-adrenoceptor activities throughout the oestrous cycle.

Methods Virgin, albino rats (Wistar strain), weighing 200-250 g, were used. The sexual state of the rat was determined by taking a vaginal smear immediately before killing by a blow to the head and bleeding out. The uterine horns were excised and 2-3 cm of the tissue from the ovarian end were set up in a 15 ml organ bath containing Tyrode solution aerated with 5% CO_2 in O_2. Uterine movements were recorded with an isotonic frontal writing lever (weight 0.5 g), moving on a smoked drum. Rats were used either in dioestrus or in oestrus, which was either natural or induced by oestradiol benzoate (0.5 mg s.c. given 3 days beforehand). After setting up the preparations, at least 1 h was allowed for the tissues to equilibrate before starting the experiment. The recordings were made at 40°, 30° and 25° C. Thirty minutes were allowed to ensure that the uterus had settled down each time the bath temperature was altered. Doses were determined for each sympathomimetic amine which gave approximately 90% of a maximal contraction, or 50% inhibition of a near maximal response to acetylcholine.

Results

Rats in dioestrus At the temperatures studied, all four amines produced solely inhibitory responses. At 40° C the mean inhibitory doses were phenylephrine (Phe) 12 μg (range, 5 to 20 μg, $n = 5$), noradrenaline (NA) 1.5 μg (0.75 to 2 μg, $n = 6$), adrenaline (Ad) 18 ng (7 to 45 ng, $n = 6$) and isoprenaline (Iso) 4 ng (2 to 6 ng, $n = 5$). As the temperature was lowered, first to 30° C and then to 25° C, although the sensitivity to acetylcholine decreased, the sensitivity of the tissue to all four sympathomimetic amines increased. At 25° C the inhibitory doses were approximately half those required at 40° C, namely Phe 6 μg (range, 3 to 10 μg, $n = 5$), NA 0.7 μg (0.5 to 1 μg, $n = 4$), Ad 11 ng (5 to 28 ng, $n = 6$) and Iso 2 ng (1 to 4 ng, $n = 5$). Spontaneous activity, which was often considerable at 40° C, was usually suppressed completely at 25° C. On returning the temperature to 40° C, the doses of the amines needed to be increased to approximately those required when the tissue was initially at this temperature to obtain the same magnitude of response. Also the spontaneous activity returned at the elevated temperature.

Rats in oestrus The responses obtained from the uteri of rats in natural and induced oestrus were

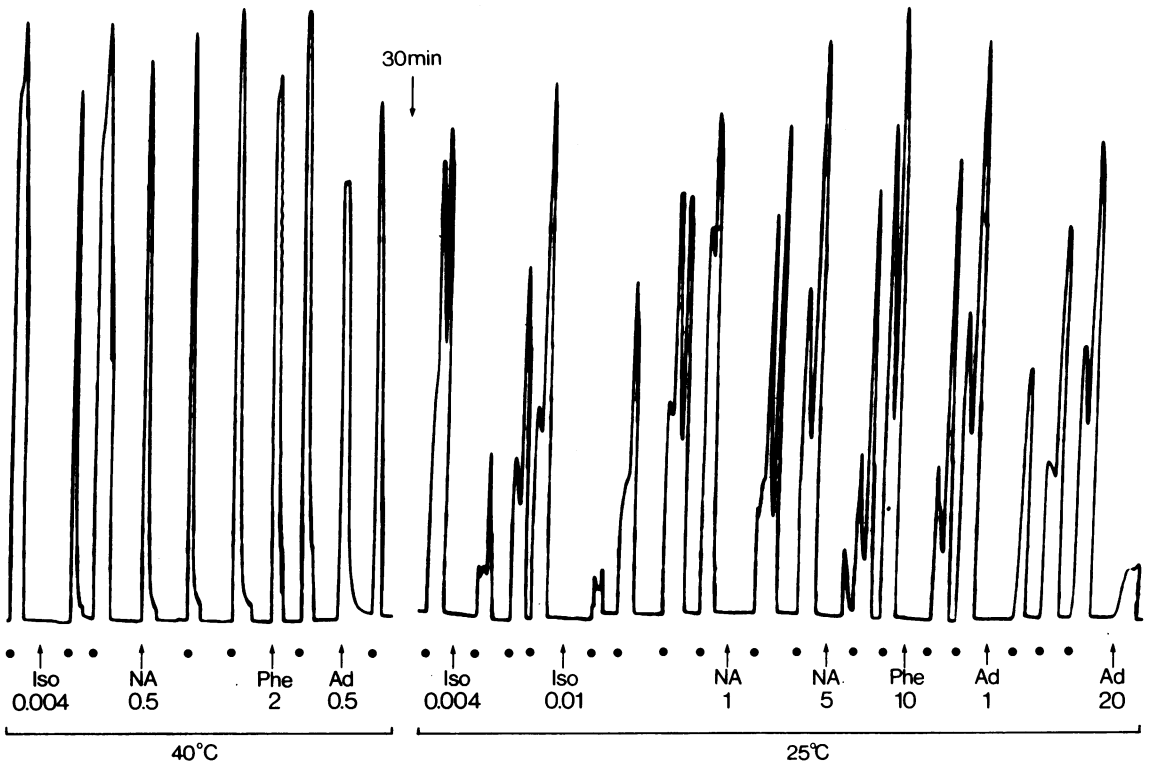

Fig. 1 Effect of lowering temperature on responses of isolated uterus from a rat in oestrus (15 ml bath). (•) Acetylcholine, 1 μg (40° C) or 2 μg (25° C). Bath solution changed with recorder stationary after each response. Doses in μg. Isoprenaline (Iso) inhibited the acetylcholine contraction more at 25° C than at 40° C. Noradrenaline (NA) and phenylephrine (Phe) were stimulant at 40° C but gave some degree of inhibition at 25° C. Adrenaline (Ad) was stimulant at 40° C; at 25° C 1 μg caused 50% inhibition but 20 μg still caused a small contraction.

similar both qualitatively and quantitatively. At 40° C NA, Ad and Phe produced contraction (which could be similar in size to that produced by acetylcholine) followed by inhibition, which was demonstrated as an anti-acetylcholine effect. These results confirm the previous work of Butterworth & Randall (1970). When the temperature of the bath was lowered to 30° C and then to 25° C the contractile responses to NA, Ad and Phe were greatly reduced or abolished depending on the dose used (Figure 1). At 40° C the mean contractile doses were NA 0.3 μg (range, 0.05 to 1 μg, $n = 9$), Ad 1.1 μg (0.1 to 5 μg, $n = 9$) and Phe 2.4 μg (1 to 5 μg, $n = 7$). When the temperature was lowered to 30° C, doses of Phe up to 100 μg ($n = 4$) produced only inhibitory responses. At this temperature the mean contractile doses for the other amines had increased to NA 2.3 μg (range, 1 to 4 μg, $n = 4$) and Ad 6.5 μg (2 to 10 μg, $n = 4$). When the temperature was lowered to 25° C, doses of Phe up to 100 μg ($n = 7$) produced only inhibitory effects. Doses of Ad up to 50 μg ($n = 6$) and NA up to 20 μg ($n = 8$) produced small contractions which usually were not greater than 10% of the height of the contractions to acetylcholine. In contrast to these effects on the α-responses, the inhibitory effects of all four amines increased as the temperature fell to 25° C. At 40° C the mean inhibitory doses were Ad 3.9 μg (range, 0.15 to 15 μg, $n = 7$) and Iso 6 ng (2 to 16 ng, $n = 5$); doses of 50 μg of Phe ($n = 6$) and 20 μg of NA ($n = 5$) not producing inhibitions greater than 20%. At 25° C the mean inhibitory doses were Phe 31 μg (range, 16 to 60 μg, $n = 7$), NA 7.3 μg (1 to 20 μg, $n = 6$), Ad 0.4 μg (0.02 to 2 μg, $n = 6$) and Iso 4 ng (3 to 7 ng, $n = 5$). When the temperature was returned to 40° C from 25° C, Ad, Na and Phe again produced near maximal contractile α-responses. Although full sensitivity was not

regained until 40° C was reached, a sudden increase in excitatory effect occurred when the temperature reached 37° C. This also coincided with the return of spontaneous activity if this had been present originally.

Discussion The experiments demonstrated that in the uterus from a rat in oestrus, where biphasic responses could be produced by several sympathomimetic amines, the initial α-excitatory phase was greatly reduced or even abolished by lowering the temperature to 25° C. On the other hand, lowering the temperature caused an increase in the inhibitory β-effects of all the amines. Thus the uterus from a rat in oestrus at the low temperature responded more like the uterus at 40° C from a rat in dioestrus, although 25° C was not low enough to counteract completely the physiological antagonism of the β- on the α-affect. Because the initial contraction produced by Ad, NA or Phe was over before any significant secondary inhibition had occurred, it seems unlikely that this influence of the β- over the α-adrenoceptors is due solely to a physiological antagonism. It should be noted that both oestrus and α-adrenoceptor activity only last for a few hours in a period of 4 to 5 days. The extreme sensitivity of α-adrenoceptors to temperature change helps to explain the failure of some workers to demonstrate contractile responses to Ad and to NA with uteri from rats in oestrus used below 37° C, a temperature at which there is a sudden decrease in contractile responses.

Iversen (1973) states that the rate of uptake of catecholamine is doubled for a 10° C rise in temperature. More amine should then be available to react with adrenoceptors at the lower temperature, tending to increase both α- and β-adrenoceptor responses. In this context it was observed that smaller doses of the amines were required at the lower temperature to produce the same degree of inhibition of the responses to acetylcholine. This factor of decreased uptake would be enough to explain the increase in the inhibitory responses at the lower temperature, but cannot explain the decreased α-responses with lowered temperature. The evidence for the involvement of prostaglandins in the production of motor responses in the isolated uterus of the rat is increasing. If it is accepted that α-responses are mediated, or at least modulated, by endogenous prostaglandins then the decreased α-responses which occur with lowered temperature could be explained by an inhibition of prostaglandin synthesis and/or activity.

The results in this paper indicate a fundamental difference between smooth muscle responses mediated by α- and by β-adrenoceptors, in that α-adrenoceptors, in contrast to β-adrenoceptors, are temperature sensitive.

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