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THE EFFECTS OF ION CHANGES ON THE CONTRACTION OF THE RAT UTERUS STIMULATED BY OXYTOCIN

BY

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The contraction of the rat uterus to oxytocin was measured when it was bathed in various solutions. Substitution of nitrate or bromide for two-thirds of the chloride present increased the size of contractions, while a similar substitution of sulphate and sucrose for chloride depressed them. Alteration of the ratio of chloride to sodium by substitution of choline for two-thirds of the sodium present enhanced the contractions, but this was probably due to the choline *per se* as choline sulphate similarly enhanced the response. Partial replacement of both sodium and chloride by sucrose did not alter the size of contractions.

The substitution of bromide, nitrate or iodide for the chloride in the bathing solution enhances the twitch tension of skeletal muscle (Hill & Macpherson, 1954) but reduces the size of isotonic contractions of cardiac muscle (Nayler & McCulloch, 1960). The effects of such anion changes on smooth muscle have not been reported, to our knowledge, but electrophysiological results suggested that such studies would be worth while. Burnstock & Straub (1958) have shown that smooth muscle is depolarized to a greater extent by potassium sulphate than by potassium chloride, and have suggested that chloride may be contributing to the membrane potential. It may be relevant in this regard that Daniel & Bass (1956) have found that there is a higher concentration of chloride in smooth muscle than in skeletal muscle. Although the role of cations in uterine smooth muscle contractility has previously been studied (see, for instance, Van Dyke & Hastings, 1928; Bentley & Dicker, 1955; and Munsick, 1960), it seemed that the effect of changes in the relative proportions of chloride and sodium would merit investigation, as Holman (1957) found with the taenia coli of the guinea-pig that tension and rate of spike discharge were increased when two-thirds of the sodium chloride in the bathing solution was replaced with choline chloride. Atropine did not abolish the effect, which was therefore presumably not due to an acetylcholine-like action of the choline. Reduction of both sodium and chloride by the use of sucrose instead of choline chloride had no such effect.

The present work reports the effects, on contractions of the rat uterus produced by oxytocin, of reducing chloride concentration in the bathing solution while leaving the sodium level unchanged, of substituting choline for sodium leaving the chloride unchanged and of replacing both sodium and chloride with either sucrose or choline sulphate.

METHODS

The uterus of the rat in oestrus was isolated and suspended in a 50 ml. bath containing a modified Locke solution (García de Jalón, Bayo Bayo & García de Jalón, 1945). The composition of this solution was: 9.0 g NaCl, 0.42 g KCl, 0.06 g $CaCl_2$, 0.5 g $NaHCO_3$, 0.5 g glucose and 1 l. water. The bath was kept at 29.5° C and gassed with oxygen. Contractions of the uterus to 3 different submaximal doses of oxytocin (Parke, Davis & Co.) were recorded while it was bathed with the modified Locke solution. The experimental solution was next run into the bath. At this stage spontaneous activity was often great. If, however, it was not sufficient to interfere with the responses to oxytocin 3 more doses identical with the previous ones were given in the same order. After 35 min this was repeated.

With the uteri that were initially contracting spontaneously, the lapse of 35 min was usually long enough for the contractions to subside, and the original 3 doses of oxytocin were again given. After the period in the experimental solution the uterus was again made to contract in the normal modified Locke solution to see whether the observed effects were reversible or not.

The results are expressed as the percentage change in response of the uterus in the experimental solution, compared to the response to the same dose of oxytocin given initially while the uterus was bathed with the standard modified Locke solution. The percentage changes with the 3 doses were pooled and averaged. The values were compared with the controls in which the modified Locke solution was used throughout the whole observational period, responses to oxytocin being measured at times corresponding to the successive tests in the experimental series.

The preliminary experiments on the permeability of the rat uterus in the various solutions are based on those of Conway (1957). Pieces of the uterus in oestrus were suspended in the experimental solutions at 2° C and weighed at intervals. The rates of swelling in the different solutions were compared to see whether there was any correlation between this rate and the contractile response.

RESULTS

Ion replacement. These results are in Table 1.

TABLE 1

THE EFFECTS OF IONIC CHANGES ON THE RESPONSE OF THE RAT UTERUS TO OXYTOCIN

The mean values (±s.e.) are given as percentage increase or decrease in response to oxytocin when de Jalon solution was replaced by experimental solution. In control experiments de Jalon solution was merely renewed.

Replacement of ⅔ NaCl in de Jalon solution by:	No. of expts.	% Increase (+) or decrease (−) in response to oxytocin: (i) Initially, in experimental solution	*P*	(ii) After 35 min in experimental solution	*P*
Sodium nitrate	10	+97·1±12·5	<0·001	+72·5±11·06	<0·01
Sodium bromide	10	+68·9±13·8	<0·001	+96·6±14·4	<0·001
Sodium sulphate +sucrose	10	−34·5± 4·3	<0·001	−57·4± 4·5	<0·001
Choline chloride	9			+100·9±15·4	<0·001
Sucrose	9			+8·1± 7·4	<0·05
Choline sulphate +sucrose	10			+142·1±17·7	<0·001
Choline bromide	10			+142·1±18·6	<0·001
Control	12	+0·7± 5·4		+27·0± 9·5	

Replacement of chloride. The sodium concentration was kept constant while the chloride was partly substituted with either nitrate or bromide. In both cases there was an immediate and reversible increase in contractions in response to oxytocin. If, however, sulphate plus sucrose was used as a substitute for chloride

there was a depression in the response which returned to normal when the sulphate plus sucrose was washed out of the bath.

Reduction of sodium concentration. The sodium concentration in the bathing solution was reduced to 0.3% while the chloride was left unchanged. Choline was substituted for the sodium. Contractions of the uterus increased markedly in response to oxytocin in this solution. Spontaneous contractions were observed initially. The increased response reverted to normal after washing out the choline. The response was unaffected by atropine sulphate (0.1 mg/l.).

In three experiments substitution of choline for all of the sodium except that present as bicarbonate (6 m-equiv/l.) resulted in spontaneous activity of the uterus and an increase in the size of the contractions produced by oxytocin, similar to that seen with $\frac{2}{3}$ substitution of choline for sodium. On returning the uterus to normal de Jalon solution, the activity returned to normal.

Reduction of sodium chloride concentration. Both the sodium and chloride of the bathing solution were reduced to $\frac{1}{3}$ the normal concentration. The reduction was effected by substitution of different solutes for the sodium chloride. If sucrose was used there was an increase in the spontaneous activity, but no change in the response to oxytocin. On the other hand, the substitution of choline sulphate plus sucrose for the sodium chloride not only increased spontaneous activity but also increased the response to oxytocin. Sucrose was used to adjust the osmotic concentration as sulphate is divalent. If choline bromide was used as a substitute for sodium chloride, there was also a marked increase in the response to oxytocin, which reverted to normal when the uterus was replaced in de Jalon solution.

Tissue swelling. The rates of swelling of the uterus when bathed in various solutions kept at 2° C are shown in Table 2. It can be seen that while there were

TABLE 2

THE RATE OF SWELLING OF THE UTERUS IN DIFFERENT SOLUTIONS

Results are given as the difference between the % swelling in the experimental solution and the % swelling in normal de Jalon solution. The figures below represent the mean ±s.e. in 6 experiments. A negative value indicates less swelling than the control. A positive one indicates more swelling. Sucrose was the only substitution that resulted in an absolute shrinkage of the uterus

Replacement of $\frac{2}{3}$ NaCl in de Jalon solution by:	1 hr	6 hr
Sodium sulphate +sucrose	−0·40±0·92	−0·74±0·92
Sodium bromide	−0·46±0·74	+1·71±0·89
Sodium nitrate	−2·18±1·28	−3·39±1·82
Sucrose	−7·37±1·10	−20·12±1·80
Choline chloride	−0·97±0·50	−6·17±1·69
Choline sulphate +sucrose	−0·02±0·94	−5·57±1·42

some marked differences in the rates of swelling in the different solutions, these were not correlated with differences in the contractile responses to oxytocin. For example, there were no significant differences between shrinkage in sodium sulphate plus sucrose and that in sodium bromide or in sodium nitrate, yet the last two enhanced the response to oxytocin while the sulphate depressed it.

It is interesting that the swelling of the pieces of uterus kept in solutions of low sodium concentration were far less than those in solutions of normal sodium concentration, which is consistent with the results of Leaf (1956) on the swelling of rat liver slices.

DISCUSSION

The present results indicate that anions play an important part in the response of uterine smooth muscle to oxytocin when the sodium concentration is unchanged. The depressant effect of sulphate might have been due to a relative impermeability of the muscle cell membrane to this ion or to a reduction in ionized calcium. The latter cannot definitely be excluded, but it may be noted that a similar increase in sulphate was not depressant in experiments with choline salts. The enhancement of contraction when $\frac{2}{3}$ of the chloride was replaced with bromide or nitrate is consistent with the results in skeletal muscle (Hill & Macpherson, 1954) but contrasts with those in cardiac muscle (Nayler & McCulloch, 1960). In skeletal muscle the increase in twitch tension seen in the presence of bromide or nitrate has been correlated with a prolonged after-potential (see Harris, 1958) and this prolonged after-potential is also seen in the presence of choline (Edwards, Ritchie & Wilkie, 1956). As the tension produced in smooth muscle is known to be related to the rate of spike discharge (Bülbring, 1958), it would be interesting to know the effects on smooth muscle of nitrate or bromide on the rate of spike discharge as well as the action potential, and see if this could be correlated with the changes in contractility.

The results with sucrose and with choline salts do not allow a clear distinction to be drawn between the possible effects of choline *per se* and of disturbances in the ratio chloride to sodium.

The enhancement of contractions with choline salts whether as chloride *or* sulphate, however, favours an effect by choline. It will be noted that this was not abolished by atropine. Possibly the membrane is more permeable to choline than sodium, but this is not indicated by the rate of swelling of the uterus in choline as compared to sodium solutions.

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