RESEARCH PAPERS

THE *IN VIVO* POTENTIATION BY MAGNESIUM SALTS OF THE UTERINE RESPONSE TO POSTERIOR PITUITARY EXTRACTS IN THE BOVINE

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INTRODUCTION

IT has been known for some years that the reactivity of isolated uterine muscle of posterior pituitary extracts varies with the magnesium ion concentration of the fluid environment. An augmenting effect of increased magnesium concentration was first demonstrated by Van Dyke and Hastings[1] using unfractionated posterior lobe extracts on the isolated guinea-pig uterus and this has since received confirmation by de Jalon[2] and Hsu[3]. Genell[4] showed that this *in vitro* phenomenon applies to species other than the guinea-pig.

Frazer[5] and Stewart[6] have studied this effect using fractionated vasopressor and oxytocic preparations. Frazer found that increase of magnesium chloride concentration can augment the response of the isolated guinea-pig uterus to either fraction but that a greater and more consistent potentiation is seen with vasopressin than with oxytocin. Stewart reinvestigated this problem over a wider range of concentrations of magnesium chloride and found potentiation of vasopressin to be greater than that of oxytocin with relatively low concentrations of magnesium chloride, but that at high concentrations oxytocin was potentiated to a greater extent than vasopressin.

The mode of action of magnesium ions in producing this augmentation is unknown. The augmentation phenomenon is known to affect uterine muscle more than other forms of smooth muscle[4]; to be inapplicable to other oxytocic drugs such as ergometrine and histamine[3,5]; and to affect vasopressin in a different way to oxytocin[5,6]. This implies a considerable degree of biochemical specificity. Genell[4] has suggested that the role of the magnesium ion as an enzyme catalyst is implicated.

Although the effect of magnesium salts is well established *in vitro* no comparable *in vivo* experiments have been traced. In the course of other work on the reactivity of the bovine uterus and cervix, to be published elsewhere, it became possible to investigate this relationship in the intact animal.

APPARATUS AND METHODS

Ten experiments were made using three cows, all of whom were non-pregnant throughout the period of observations.

Serum magnesium concentration was estimated by Allcroft's modification[7] of the method of Dennis[8]

The techniques used for recording uterine activity were essentially

similar to those used in pregnant women by Schild, Fitzpatrick and Nixon[9].

Intra uterine pressure was recorded using a hollow metal cannula carrying terminally a small latex rubber balloon (capacity 2 ml.),* the cavity of which communicated with the shaft of the cannula. This in turn was connected via pressure tubing to a mercury manometer, the movements of which were linearly magnified and recorded on a kymograph. The whole transmission system was water filled.

After sterilization, and with the balloon completely deflated, the instrument was inserted *per vaginam* until the tip lay within the body of the uterus, approximately two inches rostral to the internal cervical Os as judged by rectal examination. This position was maintained by clipping the shaft of the cannula to the vaginal protuberance of the cervix. The balloon was then partially inflated by injecting into the transmission system, a volume of water slightly less than the mould volume of the balloons.

For this procedure and throughout the subsequent experimental period the animals were maintained in the normal standing posture under extreme posterior epidural anæsthesia (segments S4 and S5) using 2·5 per cent. procaine hydrochloride.

Drugs

All drugs were given via an indwelling polythene cannula inserted into the external jugular vein.

Commercial preparations of Pitocin and Pitressin were used as sources of oxytocin and vasopressin respectively. The terms oxytocin and vasopressin when used below refer to these preparations. The manufacturers' statements, of potency and freedom from more than 5 per cent. cross contamination, were confirmed by assaying each product for both oxytocin and pressor activity.†

EXPERIMENTAL

In each experiment several doses of oxytocin, vasopressin or pituitrin were given intravenously to indicate the normal pretreatment response of the animal under prevailing conditions. Subsequently a dose of magnesium chloride or magnesium sulphate was given intravenously and the magnitude of response compared with that obtained before treatment. In some experiments the responses to both oxytocin and vasopressin were studied before and after magnesium treatment.

The observations fall into two groups. In the first, the same dose of active principle was given repeatedly throughout the experiment (Experiments 1, 4, 5, 6, 7 and 10). In the second type of experiment the dose of active principle was varied before administration of magnesium to indicate approximately the dose-response relationship over a restricted range (Experiments 2, 3, 8, 9, 10, and 11). An interval of 40 or 45

* The balloons were specially moulded by the London Rubber Co.

† These were very kindly done by G. A. Stewart of Burroughs, Wellcome and Co., Dartford.

minutes separated successive doses of hormone and this time factor precluded further elaboration of the pattern of the experiments.

RESULTS

Preliminary dosage experiments were made in which the concentration of magnesium in the blood serum was estimated before, and at intervals after, the intravenous administration of various doses of magnesium chloride. A typical experiment is illustrated in Figure 1 in which a function of the serum concentration of magnesium is plotted against time.

Treatment with magnesium chloride was found to augment uterine response of some posterior pituitary preparations but not to all. The results are summarised in Table I.

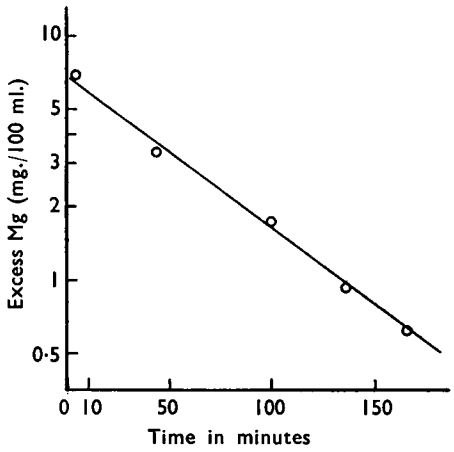

FIG. 1. Serum concentration of magnesium in a cow after the intravenous injection of 22·5 g. magnesium chloride. The logarithm of the increase in serum concentration of magnesium above the pre-injection value, is plotted against time. The slope is approximately linear.

Pituitrin. The unfractionated extract was used only in the first experiment of the series. Responses of reasonably consistent intensity were obtained when the same dose of pituitrin was given three times in succession before magnesium treatment. A fourth dose of the same magnitude, given 10 minutes after 6 g. of magnesium chloride intravenously, elicited an augmented response. This might be attributed to potentiation of either or both fractions, oxytocic and vasopressor. All subsequent experiments were made using the fractionated preparations.

Oxytocin. Uterine response to oxytocin was studied in eight experiments, in four of which vasopressin was also given. In no experiment was evidence obtained of an augmentation after magnesium even though the serum concentration was raised to 8 or 9 mg./100 ml. In most experiments (3, 5, 8, 9, 10 and 11) the treatment appeared to produce inhibition but this was not significant ($P = 0{\cdot}05$) for the group as a whole. This inhibition is shown graphically in Figure 2.

Vasopressin. Uterine responses to vasopressin were recorded in six experiments in each of which augmentation of response after magnesium was seen clearly (Fig. 2). This effect was highly significant for the group as a whole ($P = 0{\cdot}01$).

However the magnitude of the increase in response was not proportional to the corresponding serum magnesium concentration. This lack of relationship is possibly due to the discrepancy between the concentration

TABLE I

REACTIVITY OF BOVINE CORPUS UTERI BEFORE AND AFTER MAGNESIUM SALTS

Expt.	Dose	Serum concentration of magnesium in mg. per 100 ml.	Response in mm. mercury	Per cent. augmentation of response after magnesium
1	35 U. pituitrin	2·3	22	
	" " "	"	20	
	" " "	"	21	
	6 g. $MgCl_2$			
	35 U. pituitrin	3·2	29	+38
2	1 U. oxytocin	2·6	10	
	3 U. "	"	11·5	
	9 U. "	"	15	
	3 U. "	"	12	
	10 g. $MgCl_2$			
	3 U. oxytocin	5·0	12	nil
	15 g. $MgCl_2$			
	9 U. oxytocin	8·0	14	−7
3	1 U. oxytocin	2·7	5·0	
	3 U. "	"	9·5	
	9 U. "	"	15·0	
	18 g. $MgCl_2$			
	3 U. oxytocin	7·3	6·0	−37
4	3 U. oxytocin	2·7	13·5	
	3 U. "	"	14·0	
	15 g. $MgCl_2$			
	3 U. oxytocin	7·1	14·5	+3·5
5	10 U. oxytocin	2·3	15	
	10 U. "	"	13	
	4 g. $MgCl_2$			
	10 U. oxytocin	3·2	11	−15·5
6	9 U. vasopressin	2·0	10·5	
	9 U. "	"	9·0	
	5 g. $MgCl_2$			
	9 U. vasopressin	3·5	14·0	+55
7	30 U. vasopressin	3·0	34·0	
	30 U. "	"	30·0	
	30 U. "	"	39·0	
	30 U. "	"	37·5	
	20 g. $MgCl_2$			
	30 U. vasopressin	8·3	45·0	+20
8	1 U. oxytocin	3·0	3·0	
	5 U. vasopressin	"	2·0	
	2 U. oxytocin	"	4·0	
	15 U. vasopressin	"	10·0	
	6 U. oxytocin	"	15·0	
	40 U. vasopressin	"	20·0	
	23 g. $MgCl_2$			
	2 U. oxytocin	9·5	1·5	−62
	15 U. vasopressin	7·0	16·0	+60
	6 U. oxytocin	4·6	5·0	−67
9	1 U. oxytocin	3·2	5·0	
	3 U. "	"	13·0	
	18 U. vasopressin	"	20·0	
	6 U. oxytocin	"	18	
	20 g. $MgSO_4$			
	3 U. oxytocin	6·6	9·0	−18
	18 U. vasopressin	6·2	28·0	+40
	6 U. oxytocin	5·4	16·0	−11
10	9 U. vasopressin	3·0	13·0	
	2 U. oxytocin	"	15·0	
	6 U. vasopressin	"	7·0	
	2 U. oxytocin	"	12·0	
	18 g. $MgCl_2$			
	9 U. vasopressin	8·0	19·0	+46
	2 U. oxytocin	5·5	9·0	−25
	9 U. vasopressin	4·9	18·0	+40
11	3 U. oxytocin	2·4	14·0	
	6 U. "	"	32·0	
	15 U. vasopressin	"	13·0	
	30 U. vasopressin	"	24·0	
	17½ g. $MgSO_4$	"		
	3 U. oxytocin	7·8	8·0	−43
	15 U. vasopressin	4·9	18·0	+38
	6 U. oxytocin	4·6	24·0	−25
	30 U. vasopressin	3·9	30·0	+25

Doses of magnesium chloride and magnesium sulphate are calculated as anhydrous salts.

of magnesium in the blood and that in the immediate environment of the uterine cells or perhaps within the cells[10].

Comparison of responses to oxytocin and vasopressin

The differences seen between oxytocin and vasopressin in these experiments are well illustrated in the four experiments in which both oxytocin and vasopressin were given (Experiments 8, 9, 10 and 11). In all four experiments oxytocin responses showed no change or were depressed after administration of magnesium salt, whilst under the same experimental conditions responses to vasopressin were augmented. Figure 3 illustrates kymograph records from one such experiment (Experiment 8).

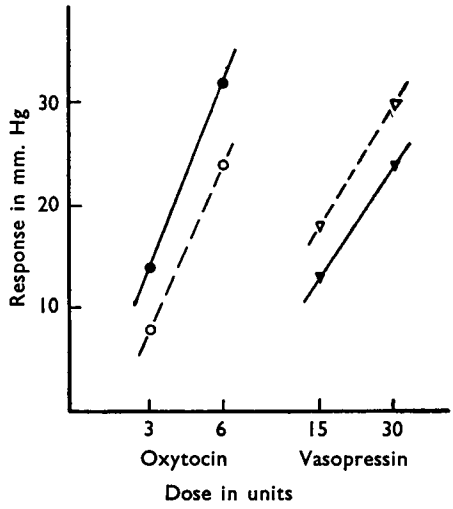

FIG. 2. Data from experiment 11. The uterine pressure response is plotted against the dose of oxytocin (circles) or vasopressin (triangles) on a logarithmic scale. Reactivity before magnesium is represented by solid symbols and continuous lines; that after magnesium by open symbols and interrupted lines. Reactivity to oxytocin is depressed after intravenous injection of 17·5 g. magnesium sulphate although at the same time reactivity to vasopressin is augmented.

DISCUSSION

Experiments of this nature suffer *in vivo* complications which make it impossible to compare the potency of posterior pituitary preparations before and after magnesium treatment, with the precision to be expected from *in vitro* experiments employing multi-dose assay procedures.

Within these limitations our results clearly indicate that an increase in the magnesium concentration of body fluids augments uterine reactivity to vasopressin, a finding in keeping with the relationship already established *in vitro* by previous workers. On the other hand our experiments showed no augmentation of oxytocin although such an effect has been detected *in vitro* by Hsu[3], Stewart[6] and others. However if the concentration of magnesium is considered, this difference is largely resolved. Frazer[5] was the first to study *in vitro* this effect over a wide range of magnesium concentrations. His results indicated progressive augmentation of vasopressin as the concentration of magnesium in the perfusion fluid increased from 2 mg. per cent. $MgCl_2$ to 5, 10, 20, 25 and 50 mg. per cent. No such augmentation was seen over this range with oxytocin and in fact depression was recorded with concentrations of 10 and 20 mg. per cent. This may be compared with the depression seen in our experiments. Stewart[6] in a somewhat similar investigation showed that at low concentrations of magnesium (2·5 and 5·0 mg per cent. $MgCl_2$)

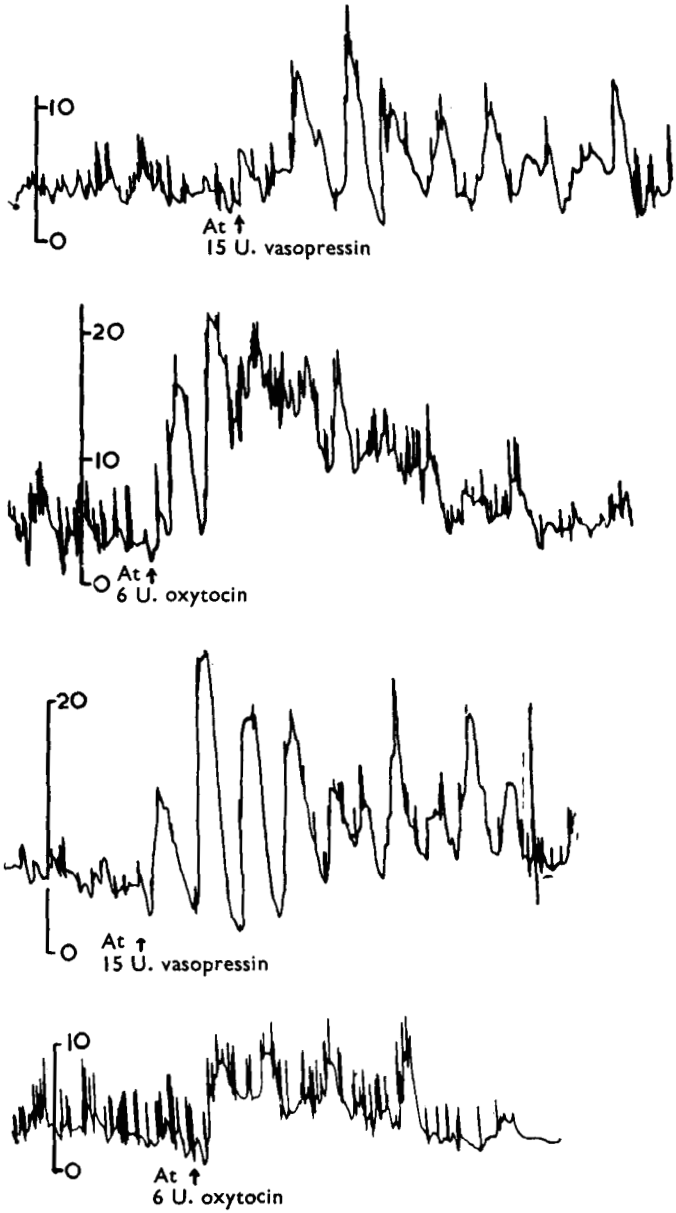

FIG. 3. Kymograph records obtained in experiment 8. Upper two tracings before, and lower two tracings after, the injection of 23 g. magnesium chloride intravenously. Before magnesium treatment the response to 6 units oxytocin is greater than that to 15 units vasopressin. Subsequent to magnesium treatment the response to the same dose of oxytocin is depressed whilst that to vasopressin is augmented, so that the vasopressin effect is the greater.

augmentation of vasopressin was predominant whereas only at high concentration (50 and 100 mg. per cent. $MgCl_2$) was oxytocin augmentation predominant.

Our *in vivo* observations were not extended to the higher concentrations used by these authors since such concentrations would certainly be toxic by virtue of central nervous system depression[11]. Similarly our lowest concentrations could not be less than those physiologically normal and it is significant that Frazer[5], Hsu[3] and Stewart[6] found evidence of magnesium augmentation of oxytocin when the concentrations studied were between zero and 2·5 mg. per cent. $MgCl_2$.

Thus the *in vitro* evidence of magnesium augmentation of oxytocin is restricted principally to concentrations of magnesium that are either too small or too large to be studied *in vivo* in experiments such as ours. Our *in vivo* evidence, of a preferential augmentation of uterine response to vasopressin consequent upon administration of magnesium salts, is in reasonable agreement with *in vitro* observations if comparison is limited to magnesium concentrations which are compatible with life.

SUMMARY

1. Intravenous injection of magnesium chloride and magnesium sulphate to intact non-pregnant bovines augments the response of the myometrium to vasopressin but not that to oxytocin. These results are discussed in relation to comparable observations *in vitro*.

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REFERENCES

1. van Dyke and Hastings, *Amer. J. Physiol.*, 1928, **83**, 563.
2. de Jalon, *Farmacoter Actual.* 1947, **4**, 177.
3. Hsu, *Quart. J. Pharm.*, 1948, **21**, 146.
4. Genell, *Skand. Arch. Physiol.*, 1938, **80**, 124.
5. Frazer, *J. Pharmacol.*, 1939, **66**, 85.
6. Stewart, *J. Pharm. Pharmacol.*, 1949, **1**, 436.
7. Allcroft, *Brit. Vet. J.*, 1947, **103**, 2.
8. Dennis, *J. biol. Chem.*, 1922, **52**, 411.
9. Schild. Fitzpatrick, and Nixon, *Lancet*, 1951, **260**, 250.
10. Martin, Mehl. and Wertman,, *Med. Clinics Nth. Amer.*, 1952, **36**, 1157.
11. Allcroft, *Brit. Vet. J.*, 1947, **103**, 157.