Effect of rubidium on responses of rabbit vas deferens to transmural stimulation

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Transmitter release is known to be very sensitive to changes in the duration of the action potential in nerve terminals (Katz & Miledi, 1967). In the present study, we have examined the influence of rubidium on adrenergic transmission in rabbit vas deferens as rubidium increased the duration of the action potential in squid giant axon (Baker, Hodgkin & Shaw, 1962).

New Zealand rabbits were killed by air embolism. Their vasa deferentia were removed and mounted longitudinally in 5 ml organ baths containing modified Krebs solution at 37° C. The medium contained 3 mM KCl and was equilibrated with 95% O_2/5% CO_2. Contractions were recorded isometrically with strain gauges. Tissues were stimulated transmurally with biphasic pulses of 1 msec duration, supramaximal voltage and 0.5-16 Hz for 60 s every 4 minutes. Transmural stimulation resulted in a rapid twitch response followed by a maintained contraction. This secondary, maintained response resulted from noradrenaline release as it was abolished by phentolamine and guanethidine. In subsequent experiments, the magnitude of the secondary response was determined.

Addition of 2 mM RbCl caused a markedly potentiated response to transmural stimulation at 2-14 Hz both in the presence and absence of 3 mM KCl in the medium. This effect was not due to an impairment of re-uptake of released noradrenaline as the 30 min uptake of [^{3}H](±)-metaraminol was not altered by 2 mM RbCl (with 3 mM KCl, uptake was 175.7 ± 11.2 pmol/g; with 5 mM KCl, 164.8 ± 17.1 pmol/g; with 3 mM KCl and 2 mM RbCl, 179.9 ± 12.8 pmol/g; mean ± s.e. of 10 observations). The responses of vasa deferentia to exogenous (−)-noradrenaline were not altered by 2 mM RbCl thus excluding an effect at receptor level or at subsequent steps in excitation-contraction coupling.

In order to determine the effect of RbCl on transmitter release, tissues were exposed to 10^{-7}M [^{3}H](±)-metaraminol for 60 minutes. After 35 min in an amine-free medium, subsequent transmural stimulation at 5 Hz for 2 min resulted in the release of [^{3}H](±)-metaraminol and this release was very significantly increased by addition of 2 mM RbCl to the medium.

These studies suggest that rubidium may potentiate adrenergic transmission by increasing the release of noradrenaline as has also been reported to occur with caesium (Johns & Paton, 1974).

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References

BAKER, P.F., HODGKIN, A.L. & SHAW, T.I. (1962). The effects of changes in ionic concentrations on the electrical properties of perfused giant axons. *J. Physiol.*, **164**, 355-374.

JOHNS, A. & PATON, D.M. (1974). Potentiating effects of caesium on transmural stimulation of rabit vas deferens. *IRCS (Med. Sci.)*., **2**, 1434.

KATZ, B. & MILEDI, R. (1967). The release of acetylcholine from nerve endings by graded electric pulses. *Proc. R. Soc. Lond. Ser. B*, **167**, 23-28.

Effects of prostaglandins in calves

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The protective effect of sodium meclofenamate against bovine anaphylaxis (Aitken & Sanford, 1972) indicated that prostaglandins might be important mediators in this species. Indomethacin also prevents the systemic hypotension, pulmonary hypertension and apnoea characteristic of anaphylaxis and inhibits Schultz-Dale contractions of isolated bovine pulmonary artery and bronchiole (Aitken & Sanford, unpublished observation).

However, Lewis & Eyre (1972) found that bovine bronchial ring failed to respond to $PGF_2\alpha$ and was relaxed by PGE_1. Effects of other prostaglandins on this tissue have not been

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described. Effects of $PGF_{2\alpha}$, $PGF_{1\alpha}$, PGE_1 and PGE_2 on the cardiovascular and respiratory systems of anaesthetized calves and on isolated pulmonary tissues have been examined in order to clarify the role of prostaglandins in bovine anaphylaxis.

Calves aged 3-4 months, anaesthetized with pentobarbitone, received prostaglandins intravenously. $PGF_{2\alpha}$ (10-60 μg/kg) increased systemic arterial B.P. by 20-85%, pulmonary arterial pressure by 20-200% and heart rate by 3-23%. Respiratory minute volume was reduced by 13-90%. $PGF_{1\alpha}$ (35-140 μg/kg) produced similar increases in systemic pressure, but had no effect on respiration. PGE_1 (1-8 μg/kg) and PGE_2 (2-8 μg/kg) reduced systemic arterial pressure (17-44%), pulmonary arterial pressure (12-37%) and heart rate (5-23%). Respiratory minute volume was reduced by PGE_1 (7-23%) and PGE_2 (26-42%). Tachyphylaxis occurred to $PGF_{2\alpha}$, $PGF_{1\alpha}$, and PGE_2.

Segments of bronchiole and spiral strips of pulmonary artery and vein were suspended in Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2 at 37° C in organ baths of 80 ml capacity. Contractions were recorded isotonically. Drugs remained in contact with artery and vein for 5 min and with bronchiole for 15 minutes.

PGF_2 contracted bronchiole (0.2-0.3 μg/ml threshold dose), pulmonary artery (0.3 μg/ml) and vein (0.1 μg/ml). All three tissues showed tachyphylaxis. $PGF_{1\alpha}$ (0.5-2 μg/ml) contracted pulmonary artery but concentrations up to 20 μg/ml had no effect on bronchiole. PGE_1 relaxed bronchiole (6-12 μg/ml) and pulmonary artery (0.5-2 μg/ml) but contracted pulmonary vein (1 μg/ml). PGE_2 had variable effects on bronchiole causing contraction (2-5 μg/ml) or, at higher concentrations (6-12 μg/ml), relaxation of muscle contracted by acetylcholine. Pulmonary artery was contracted by PGE_2 (0.4-2 μg/ml), but, when submaximally contracted by serotonin, PGE_2 (5-10 μg/ml) caused relaxation. PGE_2 (1 μg/ml) contracted vein.

Unlike Lewis & Eyre (1972), we found that $PGF_{2\alpha}$ contracted bovine bronchiole as did PGE_2. Human bronchial muscle is contracted by $PGF_{2\alpha}$ but relaxed by PGE_2 (Sweatman & Collier, 1968). Pulmonary hypertensive effects of PGE_1 and PGE_2 have been described in calves (Lewis & Eyre, 1972). The differences in our findings *in vivo* might be related to differences in depths of anaesthesia and smooth muscle tone.

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References

AITKEN, M.M. & SANFORD, J. (1972). Modification of acute systemic anaphylaxis in calves by drugs and by vagotomy. *J. comp. Path.*, **82**, 247-256.

LEWIS, A.J. & EYRE, P. (1972). Some cardiovascular and respiratory effects of prostaglandins E_1, E_2, and $F_{2\alpha}$ in the calf. *Prostaglandins*, **2**, 55-64.

SWEATMAN, W.J.F. & COLLIER, H.O.J. (1968). Effects of prostaglandins on human bronchial muscle. *Nature*, **217**, 69.

Teratogenic effects of primidone in mice

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Clinical studies have indicated that there is an increased incidence of congenital malformations, particularly cleft palate, in the infants of mothers exposed to anticonvulsant drugs during pregnancy (Speidel & Meadow, 1972). Since the majority of epileptics in these studies were treated with more than one drug it is not possible to determine exactly which anticonvulsant drugs may possess this teratogenic potential. Of the major anticonvulsants, phenytoin and phenobarbitone have both been implicated as teratogens in animals (Massey, 1969; McColl, Robinson & Globus, 1967), but we are not aware of any reports on the teratogenic effects of primidone in animals.

Primidone was administered to mice derived from the I.C.I. pathogen free strain, either in the diet or by gastric intubation for varying periods from days 6-16 of pregnancy which covers the period of embryogenesis and palatal closure in the mouse. Doses of primidone of 500, 1250, 2000 and 2500 mg/kg administered in the diet on days 6-16 of pregnancy established that primidone was teratogenic; the incidence of cleft palate increased with dose from a control incidence of 0.3% to 29.4% at the highest dose level.

As it has been suggested that low blood folate levels following anticonvulsant therapy might be related to the tetratogenic effects (Elshove, 1969), folic acid (25 mg/kg orally) was administered with primidone (1250 mg/kg). Although folic acid itself was not teratogenic, a significant ($P = 0.0004$) increase in cleft palate was seen with combined treatment compared with primidone alone. However, when folinic acid (3.75 mg/kg s.c.) was administered with primidone (1250 mg/kg) there