permeability to Ca^{2+}, or (2) extracellular Ca^{2+} is necessary to maintain intracellular Ca^{2+} stores at a normal level. In order to examine these possibilities, we have studied the action of the spasmogenic substances on ^{45}Ca uptake and efflux. ^{45}Ca uptake was determined with the La^{3+} technique (Mayer, Van Breemen & Casteels, 1972). At II 2.5×10^{-6} M, and CCh 2.5×10^{-5} M did not produce any significant change in uptake curves. On the other hand, KCl 101 mM increased ^{45}Ca uptake at 5, 10, 20 and 30 min exposure to the radioactive solution as well as the exchangeable Ca^{2+} that increased from the control value of 0.163 ± 0.007 mM/kg to 0.244 ± 0.013 mM/kg (10), $P < 0.02$. 5-HT 2.5×10^{-5} M also augmented Ca^{2+} uptake at 5, 10, 20 and 30 min, as well as the exchangeable Ca^{2+} which increased from 0.168 ± 0.009 mM/kg to 0.200 ± 0.008 (14), $P < 0.01$

Efflux curves were performed after previous equilibration with the ^{45}Ca Ringer solution. The ^{45}Ca efflux was obscured by the Ca^{2+} exchange with the binding sites in the extracellular space. In order to dissociate this latter exchange from the efflux from the cellular compartment, we displaced the Ca^{2+} bound to the extracellular and superficial membrane sites by adding 20 mM Ca^{2+} ethylene glycol-bis (β-amino-ethyl ether) N,N'-tetraacetic acid (EGTA) (Van Breemen & Casteels, 1974). Under these conditions AtII 2.5×10^{-6} M did not modify the rate of efflux of rat myometrium which varied from 13.7 ± 1.3 to 15.36 ± 0.6 $min^{-1} \times 10^{-3}$ (8), $P < 0.20$. On the contrary, efflux was augmented by 5-HT 2.5×10^{-5} M from 10.52 ± 0.6 to 16.27 ± 1.5 $min^{-1} \times 10^{-3}$ (4), $P < 0.01$; by CCh 2.5×10^{-5} M from 11.5 ± 0.9 to 14.25 ± 0.5 $min^{-1} \times 10^{-3}$ (4), $P < 0.01$ and by KCl 101 mM from 10.20 ± 0.1 to 13.70 ± 0.1 (4), $P < 0.05$. These increases of efflux may be due to a greater membrane permeability to Ca^{2+} or to the release of Ca^{2+} from intracellular binding sites.

In conclusion, AtII and CCh activate uterine smooth muscle contraction by a displacement of Ca^{2+} from intracellular stores, since Ca^{2+} uptake was not affected. The Ca^{2+} stores were in equilibrium with extracellular Ca^{2+}. On the other hand, KCl and 5-HT act at least in part by increasing the membrane permeability to Ca^{2+}.

References

VAN BREEMEN, C. & CASTEELS, R. (1974). The use of Ca . EGTA in measurements of ^{45}Ca efflux from smooth muscle. *Pflügers Arch.*, 348, 239-245.

MAYER, C.J., VAN BREEMEN, C. & CASTEELS, R. (1972). The action of lanthanum and D600 on the calcium exchange in the smooth muscle cells of the guinea-pig taenia coli. *Pflügers Arch.*, 337, 333-350.

A comparison of some smooth-muscle effects of GABA and of prostaglandin E_1

M. ISHIZAWA[1] & V.R. PICKLES

Physiology Department, University College, Cardiff CF1 1XL

The effects of GABA on intestinal smooth muscle preparations are variable, one of the most regular being inhibition of the ACh- or nicotine-induced contractions of the guinea-pig ileum (Hobbiger, 1958; Florey & McLennan, 1959). We have found direct excitatory effects from high concentrations of GABA on the guinea-pig uterus or caecal taenia. These effects may perhaps be more closely related to the depolarizing action of GABA on some nerve cells than to its hyperpolarizing action on others. The uterus and taenia also contract in response to PGE_1 in much lower concentrations; but the longitudinal muscle of the ileum or colon is less sensitive to either spasmogen.

The concentrations of GABA needed to cause contraction are generally within the range 1-30 mmol/l, but potentiation of other spasmogens has been seen in some experiments on the uterus with 0.15 mmol/l. In some experiments, the time-course of this potentiation has resembled the prolonged 'enhancement' following brief application of PGE_1 (Clegg, Hall & Pickles, 1966). At the higher concentrations, the metabolism of GABA might provide a significant amount of energy. However, the GABA-transaminase inhibitor amino-oxyacetic acid (10-45 μg/ml) neither consistently increased nor decreased the uterine responses.

Atropine (10 μg/ml) did not alter the responses of either tissue to GABA, and neither bicuculline nor its methochloride (10-25 μg/ml) clearly inhibited the responses of the uterus. Those to PGE_1, and to GABA in a few experiments, were partially inhibited by the prostaglandin-antagonist

[1] Present address: Physiology Department, Sapporo Medical College, Japan.

diphloretin phosphate (DPP, 5-10 μg/ml) but not by 7-oxa-prost-13-ynoic acid (1-10 μg/ml), the latter sometimes being spasmogenic. In these respects, the responses of the uterus both to GABA and to PGE_1 were 'atypical'.

The Demonstration consists mainly of records illustrating these statements.

We wish to thank Professor D.A. van Dorp for the PGE_1, Professor J. Fried for the 7-oxa-prost-13-ynoic acid, and Professor A.H. Jackson for the preparation of bicuculline methochloride.

References

CLEGG, P.C., HALL, W.J. & PICKLES, V.R. (1966). The action of ketonic prostaglandins on the guinea-pig myometrium. *J. Physiol., Lond.*, **183**, 123-144.

FLOREY, E. & McLENNAN, H. (1959). The effects of Factor I and of gamma-aminobutyric acid on smooth muscle preparation. *J. Physiol.*, **145**, 66-76.

HOBBIGER, F. (1958). Effects of γ-aminobutyric acid on the isolated mammalian ileum. *J. Physiol.*, **142**, 147-164.

A superfusion system used for loading and washing out ^{45}Ca from frog ventricular strips

J. STEFANOU & M.J. WOOSTER
(introduced by V.R. PICKLES)

Department of Physiology, University College, Cardiff CF1 1XL

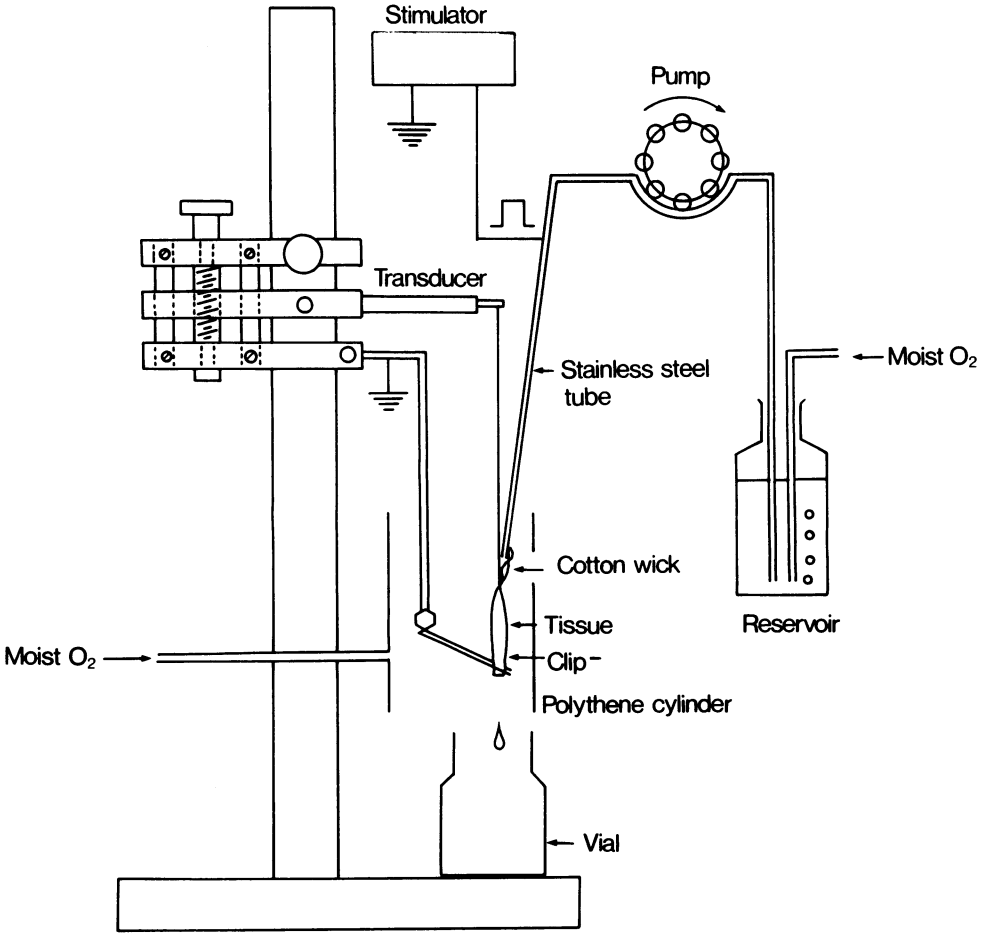

Figure 1 Diagram of superfusion system not drawn to scale. A separate recirculating circuit was used for superfusion with radioactive loading solution.