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EFFECT OF α-ADRENERGIC AGONISTS ON GLUCONEOGENESIS AND ^{45}Ca EFFLUX IN RAT KIDNEY TUBULES

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Abstract—Tubule fragments were isolated from renal cortex of fed rats and incubated with 5 mM lactate. Noradrenaline and phenylephrine (α-agonist) stimulated gluconeogenesis at all extracellular Ca^{2+} concentrations. Oxymetazoline (α-agonist) was ineffective at low extra-cellular Ca^{2+} concentrations, but stimulated the process at higher calcium concentrations. Noradrenaline stimulated ^{45}Ca efflux from prelabelled tubules. Oxymetazoline had no effect on this process. The effect of noradrenaline was blocked by thymoxamine (α-blocker) but not by propranolol (β-blocker). It is suggested that noradrenaline may promote the mobilization of an internal Ca^{2+} store whereas oxymetazoline may influence Ca^{2+} movement at the cell surface.

Gluconeogenesis in rat renal cortex can be stimulated by catecholamines [1–4]. This effect appears to be mediated through an α- rather than β-type of adrenoceptor [5, 6]. Further studies with selective agonists and antagonists have suggested that this is an α-type of receptor [7]. Renal gluconeogenesis from a number of substrates is also stimulated by addition of extracellular Ca^{2+} [8–11]. The Ca^{2+}-sensitive component (or components) in the gluconeogenesis pathway is not clearly established nor, considering the reported α-adrenergic effects on Ca^{2+} metabolism in the liver (see below), is it clear whether the α-effect on renal gluconeogenesis is mediated by altering the Ca^{2+} concentration in one or more compartments of the proximal tubule cell.

Gluconeogenesis in the liver also is stimulated by α-adrenergic action [12–16] and is generally, but not invariably, stimulated by increasing extracellular Ca^{2+} [17–23]. In the liver a considerable number of studies have presented findings implying that α-adrenergic agents cause movement of and redistribution of Ca^{2+}. Firstly, α-agonists promote efflux of total Ca^{2+} or preloaded $^{45}Ca^{2+}$ out of slices, hepatocytes or perfused liver [24–30], although Foden and Randle [31] have observed an opposite effect of α-agonists. α_1 rather than α_2 hepatic receptors appear to be involved in the promotion of Ca^{2+} release [30]. Secondly, α-agonists promote ^{45}Ca uptake by hepatocytes [25, 31, 32]. Thirdly, mitochondria isolated from liver exposed to α-agonists show a stable increase in the rate of Ca^{2+} uptake [33, 34]. Several studies have suggested that mitochondria contain the majority of the mobilizable Ca^{2+} pool that is responsive to α-adrenergic stimulation of the liver cell [35–38]. Other studies question this [28, 39].

Using cultured monkey kidney cells, Borle and Uchikawa [40] have shown that parathyrin and 3′5′cyclic AMP [41] increase ^{45}Ca uptake, total cell Ca^{2+} and exchange of Ca^{2+} between cellular pools. However, there have been no studies of the effects of catecholamines upon Ca^{2+} in the renal cortex. It was, therefore, the purpose of this study to investigate the effects of α-agonists on $^{45}Ca^{2+}$ uptake by and efflux from tubule fragments isolated from rat renal cortex. Both noradrenaline and the synthetic imidazoline agonist oxymetazoline were used as agonists. Although classified as an α-agonist, oxymetazoline differs in action from noradrenaline in this system in a number of respects [6, 42, 43]. In particular, unlike noradrenaline, oxymetazoline does not stimulate gluconeogenesis from pyruvate in the absence of extracellular Ca^{2+} or in the presence of low Ca^{2+} concentrations [6]. A part of this study is devoted to further investigation of this noteworthy finding.

MATERIALS AND METHODS

Chemicals. These were obtained and treated as described previously [6, 7, 44]. In addition, phenylephrine was from Sigma (London) Chemical Co. (Kingston-upon-Thames, U.K.) $^{45}CaCl_2$ was from the Radiochemical Centre (Amersham, U.K.) and 2,5-bis-(5-*t*-butylbenzoxazol-2-yl) thiophen from CIBA (A.R.L) Ltd. (Duxford, U.K.).

Animals. These were male Sprague–Dawley rats bred in the animal colony at University College London. They were maintained on GR3EK diet (E. Dixon and Sons, Ware, U.K.) with unlimited access to water until the time of experimentation when they weighed 160–180 g.

Isolation of tubule fragments from renal cortex. This was as described by Macdonald and Saggerson [6] and is performed in Krebs–Ringer bicarbonate buffer [45] containing 1.27 mM Ca^{2+}, collagenase (2 mg/ml) and fatty acid-poor albumin (20 mg/ml). When tubules were isolated for study of ^{45}Ca efflux, $^{45}CaCl_2$ (2 μCi/ml) was also added to this

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medium, i.e. a ^{45}Ca loading procedure was combined with disaggregation of the tissue. As described previously [6], collagenase treatment was followed by filtration and washing by centrifugation at 100 g_{av} for 1 min. This was performed twice using 10 ml of Krebs–Ringer bicarbonate containing 1.27 mM Ca^{2+} and fatty acid-poor albumin (10 mg/ml) to wash the tubules from 4 to 6 kidney cortices. When glucose production was to be measured the tubules were finally made up in a stock suspension in which the tissue from each original kidney cortex was dispersed in 2.5 ml of Krebs–Ringer bicarbonate containing 1.27 mM Ca^{2+} and fatty acid-poor albumin (10 mg/ml). When ^{45}Ca efflux was to be measured the stock suspension contained double this content of tubular material.

Incubation techniques. Incubations were commenced immediately after the preparation of tubule fragments by dispensing 0.5 ml portions of the stock suspension into 25 ml silicone-treated Erlenmeyer flasks which finally contained 5 ml of Krebs–Ringer bicarbonate buffer with fatty acid-poor albumin (10 mg/ml), 5 mM sodium L-lactate and the appropriate additions of agonists and antagonists. The final calcium concentration in incubation media is indicated in the figures and tables. Incubation was at 37° with shaking at approx. 70 oscillations/min. The flask contents were continuously gassed with O_2/CO_2 (19:1).

Analytical methods. Glucose was measured enzymically [46] in neutralised extracts prepared after deproteinisation of entire flask contents or of 1.0 ml samples taken from flasks using $HClO_4$ [6]. In all experiments the small amount of glucose initially present in non-incubated preparations was also determined and subtracted from experimental values.

^{45}Ca efflux during incubations was measured by taking 0.2 ml samples from tubule incubations and plunging these rapidly into 0.8 ml of ice-cold Krebs–Ringer bicarbonate buffer containing albumin (10 mg/ml) and 2.5 mM $LaCl_3$ in a plastic tube. The tube was shaken vigorously for approx. 5 sec and then centrifuged for 30 sec on a Beckman 5412 centrifuge. The resulting supernatant was immediately decanted off and stored in ice until the end of the experiment when a 0.5 ml sample was taken for liquid scintillation counting in 10 ml of scintillation fluid consisting of 2,5-bis-(5-*t*-butylbenzoxazol-2-yl) thiophen (4 g/l) in toluene + Triton X-100 (2:1 parts by volume). The efficiency of counting in a Beckman Ls-355 scintillation counter was 79–82%.

The DNA content of portions of the stock tubule suspensions was measured by the method of Burton [47].

Statistical methods. Analysis of data was performed on a paired basis and statistical significance determined by the Student's *t*-test.

RESULTS AND DISCUSSION

Stimulation of gluconeogenesis by α-agonists with varying extracellular [Ca^{2+}]

Following the finding of Macdonald and Saggerson [6] that oxymetazoline is ineffective at low [Ca^{2+}] whereas noradrenaline stimulated gluconeogenesis from pyruvate in the absence of Ca^{2+} (100 μM EGTA present), it was decided to investigate the effect of these agonists over a wide range of [Ca^{2+}] (Table 1, Fig. 1). The effect of another synthetic α-agonist,

Table 1. Effect of extracellular Ca^{2+} concentration upon stimulation of gluconeogenesis by noradrenaline, phenylephrine and oxymetazoline

Agonist used	[Ca^{2+}] (mM)	Glucose production (μmole/30 min per mg DNA) Basal	With agonist	Increase due to agonist	Number of experiments	'P' for comparison of agonist-treated vs basal
Noradrenaline (1 μM)	0.13	0.31 ± 0.07	0.68 ± 0.12	0.38 ± 0.08	6	< 0.01
	0.20	0.40 ± 0.08	0.84 ± 0.12	0.44 ± 0.09	6	< 0.01
	0.50	0.79 ± 0.14	1.30 ± 0.17	0.51 ± 0.08	6	< 0.01
	1.0	1.38 ± 0.14	2.06 ± 0.26	0.68 ± 0.16	6	< 0.01
	2.0	1.97 ± 0.25	2.41 ± 0.36	0.44 ± 0.19	6	< 0.1
	4.5	2.26 ± 0.36	2.71 ± 0.25	0.50 ± 0.12	5	< 0.01
Phenylephrine (10 μM)	0.13	0.49 ± 0.08	0.91 ± 0.15	0.42 ± 0.10	7	<0.01
	0.50	0.90 ± 0.14	1.80 ± 0.30	0.89 ± 0.24	7	<0.01
	1.0	1.78 ± 0.24	2.84 ± 0.35	1.01 ± 0.23	6	<0.01
	2.0	2.82 ± 0.34	3.62 ± 0.38	0.80 ± 0.17	7	<0.01
	4.5	2.96 ± 0.32	3.39 ± 0.35	0.43 ± 0.22	6	<0.2
Oxymethazonline (10 nM)	0.13	0.66 ± 0.11	0.75 ± 0.18	0.10 ± 0.14	5	< 0.5
	0.20	0.68 ± 0.17	1.03 ± 0.19	0.35 ± 0.10	5	< 0.05
	0.50	1.09 ± 0.20	1.67 ± 0.36	0.58 ± 0.19	5	< 0.05
	1.0	1.74 ± 0.39	2.78 ± 0.63	1.04 ± 0.29	5	< 0.05
	2.0	2.78 ± 0.59	3.98 ± 0.99	1.20 ± 0.43	4	< 0.1
	4.5	3.63 ± 0.88	4.15 ± 0.83	0.52 ± 0.36	5	< 0.3

Tubule fragments were prepared and incubated with 5 mM sodium lactate as described under Materials and Methods. Samples were taken for analysis at 15 and 45 min. The values are the increments in glucose production in this 30 min interval and are means ± S.E.M. The mean tubule DNA per ml of flask contents was: noradrenaline experiments, 48 μg; phenylephrine experiments 38 μg; oxymetazoline experiments 27 μg.

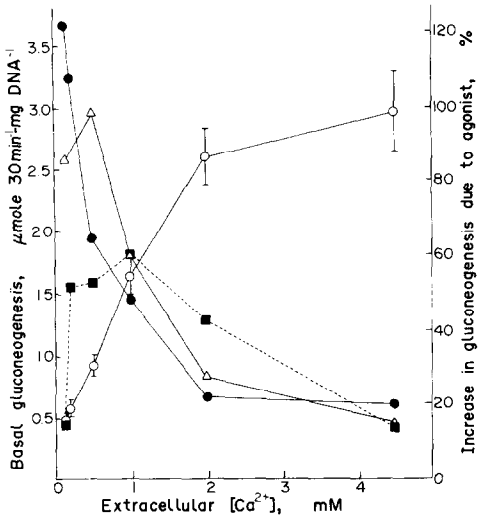

Fig. 1. Effect of extracellular Ca^{2+} concentration upon percentage stimulation of gluconeogenesis by noradrenaline, phenylephrine and oxymetazoline. The values are derived from Table 1: ●, effects of noradrenaline; △, effects of phenylephrine; ■, effects of oxymetazoline; ○, means ± S.E.M. of basal rates of glucose production (18 experiments).

phenylephrine, was also examined. The experimental design differed from that of Macdonald and Saggerson [6]. Firstly lactate was used as substrate since both oxymetazoline [6] and noradrenaline [5] give greater stimulation of gluconeogenesis with this substrate than with pyruvate. Second, as described by Saggerson [11], tubules were not Ca^{2+}-depleted in the course of isolation, $[Ca^{2+}]$ was adjusted as required at the start of incubations and measurements of glucose production were made at 15 and 45 min. Over this time interval glucose formation from pyruvate [11] or lactate, (results not shown) is linear with time. Any transient non-linear portions of the time-course of glucose output resulting from adjustment of $[Ca^{2+}]$ or addition of agonists occurs within this initial 15 min period. The rates of glucose production shown in Tables 1 and 2 and Figs. 2 and 3, therefore, represent steady state rates. The concentrations of agonists were those found to give maximum stimulation of gluconeogenesis at 1.27 mM Ca^{2+} under these experimental conditions (see Figs. 2 and 3). It may be seen that oxymetazoline differed from noradrenaline and phenylephrine in that it was almost ineffective at 0.13 mM Ca^{2+} and less effective than the other agonists at 0.2 mM Ca^{2+}. All the agonists were generally similar in effectiveness at and above 1 mM Ca^{2+}. These differences are seen more clearly when the data of Table 1 are transformed into percentage changes (Fig. 1). At 0.13 mM Ca^{2+}, noradrenaline and phenylephrine increased glucose production by 129 ± 25 and 93 ± 24%, respectively. These percentage increases were significantly different ($P < 0.01$) for noradrenaline and $P < 0.05$ for phenylephrine) from the percentage increase of 15 ± 22% seen with oxymetazoline at this low $[Ca^{2+}]$. Inclusion of the β-blocker propranolol (10 μM) did not alter the effect of noradrenaline at any tested $[Ca^{2+}]$ (results not shown). Therefore, the differences between noradrenaline and oxymetazoline could not be explained by the possibility that, at low $[Ca^{2+}]$, noradrenaline might stimulate gluconeogenesis through β-adrenoceptors. Since a wide range of $[Ca^{2+}]$ was covered, it was necessary, for technical reasons, to use separate tubule preparations for the studies with each of the three agonists shown in Table 1. It is probably for this reason that

Table 2. Effect of low extracellular Ca^{2+} concentrations upon stimulation of gluconeogenesis by noradrenaline, phenylephrine and oxymetazoline

Ca^{2+}	Basal	+ Agonist		
		noradrenaline (1μM)	phenylephrine (10μM)	oxymetazoline (10nM)
0.13 mM Glucose production (μmole/30 min per mg DNA)	0.72 ± 0.13	1.20 ± 0.15*	1.00 ± 0.16†	0.74 ± 0.15
Difference from basal		0.48 ± 0.05‡	0.29 ± 0.04§	0.02 ± 0.02
% Difference from basal		+86 ± 17§	+48 ± 12‖	+2 ± 2
0.2 mM Glucose production (μmole/30 min per mg DNA)	0.83 ± 0.16	1.39 ± 0.23†	1.20 ± 0.18†	0.98 ± 0.18†
Difference from basal		0.56 ± 0.09‖	0.37 ± 0.08	0.15 ± 0.03
% Difference from basal		+72 ± 11§	+49 ± 10¶	+19 ± 3

Tubule fragments were prepared and incubated with 5 mM sodium lactate as described under Materials and Methods. Samples were taken for analysis at 15 and 45 min. The values are the increments in glucose production in this 30 min interval and are means ± S.E.M. of 5 experiments. The mean tubule DNA per ml of flask contents was 51 μg.

* $P < 0.001$, † $P < 0.01$ vs the basal condition.

‡ $P < 0.001$, § $P < 0.01$

‖ $P < 0.02$, ¶ $P < 0.05$ for comparison of the effect of oxymetazoline vs the effects of noradrenaline or phenylephrine.

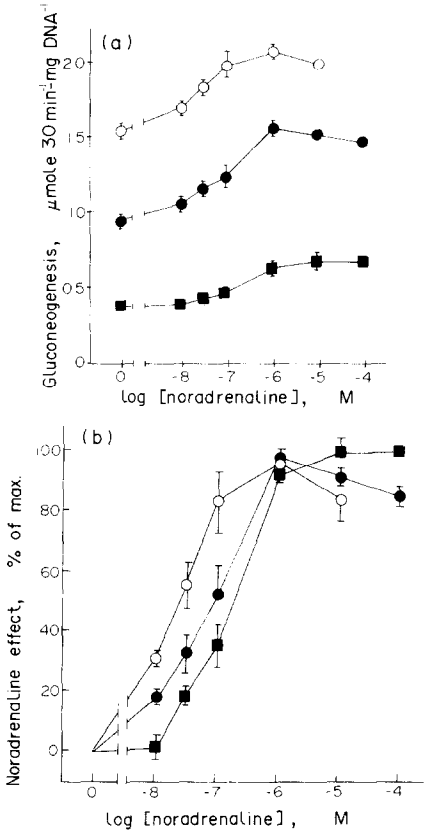

Fig. 2. Effect of extracellular Ca^{2+} concentration upon the dose-dependence of stimulation of gluconeogenesis by noradrenaline. Tubule fragments were prepared and incubated with 5 mM lactate as described under Materials and Methods. Samples were taken for analysis at 15 and 45 min. Ca^{2+} concentrations were: ■, 0.13 mM; ●, 0.7 mM; ○, 1.27 mM. The values are means of 4 experiments. The mean tubule DNA content was 38 μg per ml of flask contents. (a) The values are expressed in absolute terms as the increment in glucose production between 15 and 45 min. The bars indicate S.E.M. and, where not shown, like within the symbol. (b) The values are normalized by expressing the basal value as zero and the maximum value in each experiment as 100. Individual increases due to noradrenaline are then expressed as percentages of the largest increase in each experiment. The bars indicate S.E.M. Significant differences were seen between the effects at 0.13 mM and 1.27 mM Ca^{2+} when the noradrenaline concn. was 10, 30 and 100 nM ($P < 0.001$, < 0.001, < 0.01, respectively). The effect seen with 10 nM noradrenaline at 0.7 mM Ca^{2+} was significantly different ($P < 0.01$) from that seen at 0.13 and 1.27 mM Ca^{2+} with this concentration of hormone.

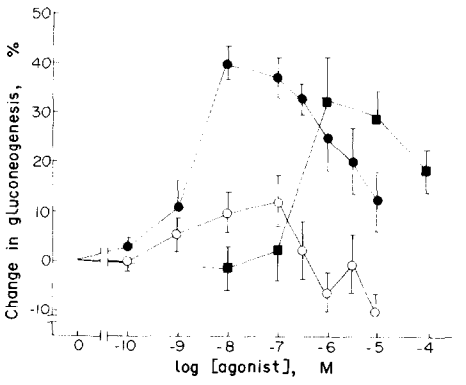

Fig. 3. Dose-dependence of stimulation of gluconeogenesis by oxymetazoline and phenylephrine. Tubule fragments were prepared and incubated with 5 mM lactate as described under Materials and Methods. Samples were taken for analysis at 15 and 45 min. The values are calculated from the increments in glucose production in this 30 min interval and are means ± S.E.M. of 4 experiments in each case. The mean tubule DNA per ml of flask contents was 47 μg for oxymetazoline experiments and 57 μg for phenylephrine experiments. Basal rates of gluconeogenesis (μmole/30 min per mg DNA) were: in oxymetazoline experiments, 0.53 ± 0.09 (Ca^{2+} = 0.13 mM) and 2.12 ± 0.18 (Ca^{2+} = 1.27 mM); phenylephrine experiments, 1.76 ± 0.22 (Ca^{2+} = 1.27 mM). ●, with oxymetazoline, [Ca^{2+}] = 1.27 mM; ○, with oxymetazoline [Ca^{2+}] = 0.13 mM; ■, with phenylephrine [Ca^{2+}] = 1.27 mM.

the percentage effects of oxymetazoline and noradrenaline were not significantly different at 0.2 mM Ca^{2+} in this experiment. However, this was seen in a further, more closely matched experiment. Table 2 summarises an experiment in which the actions of the three agonists were examined in the same tubule preparations over a more limited range of [Ca^{2+}]. The results demonstrate a small, but significant effect of oxymetazoline at 0.2 mM Ca^{2+} (19%) but this was significantly less than the effects of noradrenaline (72%) or phenylephrine (49%). Although noradrenaline is effectve in all [Ca^{2+}] when used at the relatively high dose of 1 μM, lowering the extracellular [Ca^{2+}] reduced the effectiveness of low concentrations of noradrenaline (Fig. 2); i.e. on changing from 1.27 to 0.13 mM Ca^{2+} the percentage of the maximal effect obtained with 10 nM, 30 nM and 100 nM noradrenaline decreased from 31 ± 2, 55 ± 8 and 83 ± 10% to 2 ± 4, 19 ± 3 and 35 ± 7%, respectively (Fig. 2b). From Fig. 2b it may be seen that decreasing [Ca^{2+}] from 1.27 to 0.13 mM increased the half-maximally effective concentration of noradrenaline by approximately 6-fold (from 2.7×10^{-8} M to 1.7×10^{-7} M). Lowering extracellular [Ca^{2+}] has also been shown to reduce the effectiveness of low concentrations of adrenaline to stimulate renal gluconeogenesis from pyruvate (see Fig. 2 of [44]). In addition, it is noteworthy that Ca^{2+} depletion of rat hepatocytes also causes a shift to the right in the dose-curve for activation of phosphorylase by adrenaline [48]. In a similar experiment (Fig. 3) it was found that the effectiveness of oxymetazoline as an agonist at 0.13 mM Ca^{2+} could not be restored by raising its concentration. Hence, the loss of response to oxymetazoline seen at low [Ca^{2+}] is not the result of a dose-curve shift of the type seen in Fig. 2b.

In Fig. 3 significant effects of oxymetazoline were observed at 10 nm, 0.1 μM, 0.3 μM and 1 μM ($P < 0.05$ or less) when the [Ca^{2+}] was 1.27 mM. The small effects of this agonist seen at 0.13 mM Ca^{2+} were in every case non-significant.

In conclusion, these experiments show that the lowering of extracellular [Ca^{2+}] abolishes stimulation of gluconeogenesis by oxymetazoline and by low

concentrations of noradrenaline. Higher concentrations of noradrenaline (or phenylephrine) however are still effective in this respect.

Effects of α-agonists on ^{45}Ca *efflux*

A series of experiments were undertaken in which ^{45}Ca-preloaded tubule fragments were incubated in 1.27 mM Ca^{2+} with high concentrations of noradrenaline (1 μM) or oxymetazoline (10 nM). Efflux of ^{45}Ca in the absence of agonists is shown in Figs. 4 and 5. Under these basal conditions 50% of the ^{45}Ca taken up during the loading phase appeared in the incubation medium in 20–25 min. This time-course of basal efflux of ^{45}Ca was reproducibly seen. Noradrenaline increased the efflux of ^{45}Ca (Fig. 4) the effect being statistically significant ($P < 0.05$ or less) at all the shown times except at 15 min ($P < 0.1$). The differences between the basal efflux and that in the presence of noradrenaline increased with time in an approximately linear fashion (Fig. 4). In contrast, oxymetazoline, under conditions where this agonist is effective in promoting gluconeogenesis, was total ineffective in promoting ^{45}Ca efflux (Fig. 5). The effect of noradrenaline on ^{45}Ca efflux was mediated through α-rather than β-adrenoceptors since the α_1 antagonist thymoxamine (which blocks stimulation of renal gluconeogenesis by noradrenaline [7], but not propranolol abolished the effect of noradrenaline on ^{45}Ca efflux (Table 3). The effect of noradrenaline on ^{45}Ca efflux in this system (Fig. 4) was considerably smaller than the effect of

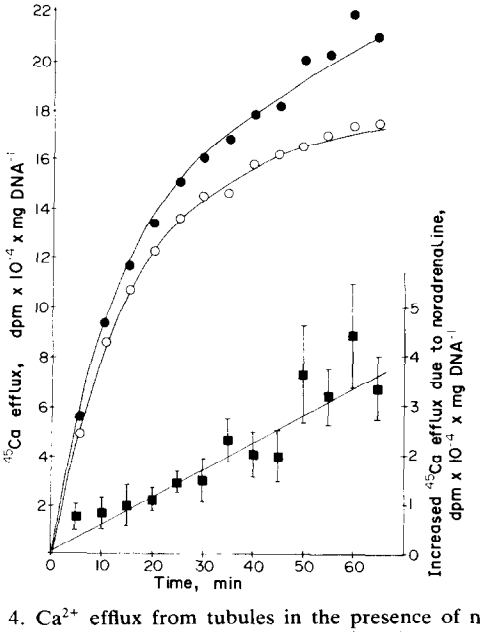

Fig. 4. Ca^{2+} efflux from tubules in the presence of noradrenaline. Tubule fragments, prepared and preloaded with ^{45}Ca as described under Materials and Methods, were incubated with 5 mM sodium L-lactate and 1.27 mM Ca^{2+}. Samples were taken for measurements of ^{45}Ca efflux at the indicated times. The zero-time value was subtracted from these values. The values are the means of 8 experiments in which the mean tubule DNA per ml of flask contents was 96 μg. ○, ●, ^{45}Ca efflux in the absence and presence, respectively of 1 μM noradrenaline; ■, means ± S.E.M. of differences between these values.

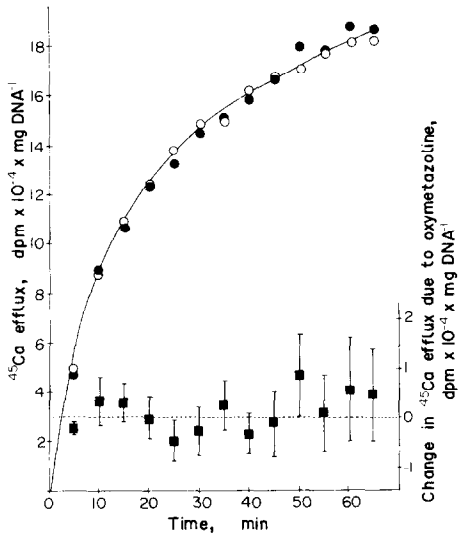

Fig. 5. Ca^{2+} efflux from tubules in the presence of oxymetazoline. Tubule fragments, prepared and preloaded with ^{45}Ca as described under Materials and Methods, were incubated with 5 mM sodium L-lactate and 1.27 mM Ca^{2+}. Samples were taken for measurement of ^{45}Ca efflux at the indicated times. The zero-time value was subtracted from these values. The values are the means of 7 experiments in which the mean tubule DNA per ml of flask contents was 95 μg. ○, ●, ^{45}Ca efflux in the absence and presence respectively of 10 nM oxymetazoline; ■, means ± S.E.M. of differences between these values.

phenylephrine on this process in rat hepatocytes [25]. This could imply a difference in the scale of this effect between the two tissues. Alternatively, the tubule fragment preparation is a heterogeneous cell population and a proportion of the basal efflux is likely to be from cells or structures which do not respond in this fashion to α-adrenergic stimulation.

Effects of α-agonists on ^{45}Ca *uptake*

Keppens *et al.* [32] and Assimacopolous-Jeanett *et al.* [25] showed that phenylephrine stimulates uptake of ^{45}Ca by rat hepatocytes at early times (1–5 min). A similar series of experiments were undertaken with tubule fragments (results not shown). These were incubated with ^{45}Ca and [^{3}H]inulin as extracellular marker and ^{45}Ca uptake determined after centrifugation of tubules through a layer of silicone oil. Although rapid uptake of ^{45}Ca was observed these experiments were negative in that neither noradrenaline (1 μM) nor oxymetazoline (10 nM) had any effect on ^{45}Ca uptake over a period of 1–10 min. Because of the heterogenicity of the tissue preparation it cannot be established whether these agonists do not stimulate ^{45}Ca uptake or whether basal ^{45}Ca uptake into α-agonist-insensitive cells is sufficiently large to mask a stimulation of this process.

General discussion

This study shows two clear differences between the α-adrenergic effects of noradrenaline and oxymetazoline in the renal tubule; namely, an inability

Table 3. Effect of propranolol and thymoxamine upon the stimulation of ^{45}Ca efflux by noradrenaline

Experiment No.	Additions to incubation	^{45}Ca efflux (dpm × 10^{-4}/mg DNA) 30 min	60 min
1	Propranolol (10μM)	11.0 ± 0.9	13.9 ± 1.0
	Propranolol (10μM)† noradrenaline (1μM)	12.6 ± 0.9*	16.3 ± 1.1†
2	Thymoxamine (10μM)	11.4 ± 0.9	14.9 ± 0.7
	Thymoxamine (10μM) + noradrenaline (1μM)	11.5 ± 0.8	14.2 ± 1.3

Tubule fragments, prepared and preloaded with ^{45}Ca as described under Materials and Methods, were incubated with 5 mM sodium L-lactate and 1.27 mM Ca^{2+}. Samples were taken for measurement of effluxed ^{45}Ca at the indicated times. The zero-time value was subtracted from these values which are the means ± S.E.M. from 6 separate tubule preparations in both cases. The mean tubule DNA per ml of flask contents was: experiment 1, 80 μg; experiment 2, 80 μg. Significance of noradrenaline effects are indicated by *, † = $P < 0.01$, < 0.02, respectively.

of oxymetazoline to promote ^{45}Ca efflux or to stimulate gluconeogenesis at low extracellular [Ca^{2+}]. These are in addition to other differences noted previously, i.e. oxymetazoline does not stimulate gluconeogenesis from succinate [6], its action is less inhibited by ouabain [42] and is more sensitive to blockade by D-600 (methoxyverapamil) and phentolamine [43]. The reasons underlying this apparent subdivision of α-agonist action are unknown at present. Curiously, although gluconeogenesis in hepatocytes is stimulated by noradrenaline or phenylephrine, oxymetazoline does not appear to stimulate the process in this tissue [15].

Obviously, ^{45}Ca flux experiments must be interpreted with considerable caution. The increased efflux of ^{45}Ca in the presence of noradrenaline could be due to mobilization of Ca^{2+} from an internal store resulting in increased exchange of ^{45}Ca with the incubation medium. If this is so, then clearly oxymetazoline does not promote the mobilization of this calcium store.

As discussed by Jenkinson *et al.* [49] α-receptor mediated events in various experimental systems are dependent upon extracellular Ca^{2+}; e.g. the slow phase of contraction of arterial smooth muscle, the inhibitory effect upon guinea pig taenia coli; the sustained phase of K^+ release from the parotid. In these cases it is inferred that α-adrenergic stimulation is associated with a change in Ca^{2+} permeability of the plasma membrane. On the other hand other α-receptor-promoted events can be observed in the absence of or presence of only very low extracellular Ca^{2+}; e.g. glucose release from liver (see above for references), the rapid initial phase of the contractile response of arterial smooth muscle, the early transient phase of K^+ release from the parotid (see [49]). It is presumed that these latter events are achieved by mobilization of internal Ca^{2+} stores.

It is tentatively suggested that the action of oxymetazoline in renal cortex is primarily concerned with a pool or store of Ca^{2+} at or near the cell surface. This may be extremely difficult to establish. On the other hand it is suggested that part at least of the action of noradrenaline is concerned with the disposition of an internal store of Ca^{2+}.

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REFERENCES

1. D. Friedrichs and W. Schoner, *Biochim. biophys. Acta* **304**, 142 (1973).
2. S. Klahr, T. Nawar and A. C. Schoolworth, *Biochim. biophys. Acta* **304**, 161, (1973).
3. K. Kurokawa and S. G. Massry, *J. clin. Invest* **52**, 961, (1973).
4. A. Roobol and G. A. O. Alleyne, *Biochem. J.* **134**, 157 (1973).
5. W. G. Guder and A. Rupprecht, *Eur. J. Biochem.* **52**, 283, (1975).
6. D. W. R. MacDonald and E. D. Saggerson, *Biochem. J.* **168**, 33 (1977).
7. P. Kessar and E. D. Saggerson, *Biochem. J.* **190**, 119 (1980).
8. H. A. Krebs, D. A. H. Bennet, P. du Gasquet, T. Gascoyne and T. Yoshida, *Biochem. J.* **86**, 22 (1963).
9. J. Z. Rutman, L. E. Meltzer, J. R. Kitchell, R. J. Rutman and P. George, *Am. J. Physiol.* **208**, 841 (1965).
10. N. Nagata and H. Rasmussen, *Biochim. biophys. Acta* **215**, 1, (1970).
11. E. D. Saggerson, *Biochem. J.* **179**, 131, (1978).
12. N. M. Kneer, A. L. Bosch, M. G. Clarke and H. A. Lardy, *Proc. natn. Acad. Sci. U.S.A.* **71**, 4523 (1974).
13. J. H. Exton and S. C. Harper, *Adv. cyclic Nucleotide Res.* **5**, 519 (1975).
14. N. J. Hutson, F. T. Brumley, F. D. Assimacopoulos, S. C. Harper and J. H. Exton, *J. biol. Chem.* **251**, 5200, (1976).
15. J. C. Garrison and M. K. Borland, *J. biol. Chem.* **254**, 1129, (1979).
16. N. B. Kneer, M. J. Wagner and H. A. Lardy, *J. biol. Chem.* **254**, 12160 (1979).
17. N. Friedmann and H. Rasmussusen, *Biochem. biophys. Acta* **222**, 41 (1970).
18. R. N. Zahlten, F. W. Stratman and H. A. Lardy, *Proc. natn. Acad. Sci. U.S.A.* **70**, 3213 (1973).
19. R. N. Zahlten, N. M. Kneer, F. W. Stratman and H. A. Lardy, *Archs Biochem. Biophys.* **161**, 528, (1974).
20. M. E. M. Tolbert and J. N. Fain, *J. biol. Chem.* **2119**, 1162 (1974).
21. S. J. Pilkis, J. H. Claus, R. A. Johnson and C. R. Pakr, *J. biol. Chem.* **250**, 6238 (1975).

22. K. R. F. Elliot, *FEBS Lett.* **64**, 62 (1976).
23. R. Rognstad, *Int. J. Biochem.* **7**, 403 (1976).
24. D. G. Haylett, *Br. J. Pharmac.* **57**, 158 (1976).
25. F. D. Assimacopolous-Jeannet, P. F. Blackmore and J. H. Exton, *J. biol. Chem.* **252**, 2662 (1977).
26. P. F. Blackmore, F. T. Brumley, J. L. Marks and J. H. Exton, *J. biol. Chem.* **253**, 4851 (1978).
27. J.-L. J. Chen, D. F. Babcock and H. A. Lardy, *Proc. natn. Acad. Sci. U.S.A.* **75**, 2234 (1978).
28. M. Althaus-Salzmann, E. Carafoli and A. Jacob, *Eur. J. Biochem.* **106**, 241, (1980).
29. J. P. Demaye, P. F. Blackmore, J. C. Venter and J. H. Exton, *J. biol. Chem.* **255**, 3905 (1980).
30. P. F. Blackmore and J. H. Exton, *Biochem. J.* **198**, 370 (1981).
31. S. Foden and P. J. Randle, *Biochem. J.* **170**, 615 (1978).
32. S. Keppens, J. R. Vandenheede and H. Dewulf, *Biochim. biophys. Acta* **304**, 161 (1977).
33. L. M. Taylor, V. Prpic, J. H. Exton and F. L. Bygrave, *Biochem. J.* **188**, 443 (1980).
34. P. H. Reinhard, W. M. Taylor and F. L. Bygrave, *FEBS Lett.* **120**, 71 (1980).
35. D. F. Babcock, J.-L., J-Chem, B. P. Yip and H. A. Lardy, *J. biol. Chem.* **254**, 8117 (1979).
36. P. F. Blackmore, J. P. Dehaye, W. G. Strikland and J. H. Exton, *FEBS Lett.* **100**, 117 (1979a).
37. P. F. Blackmore, J. H. Dehaye and J. H. Exton, *J. biol. Chem.* **254**, 6945 (1979b).
38. E. Murphy, K. Coll, T. L. Rich and J. R. Williamson, *J. biol. Chem.* **255**, 6600 (1980).
39. J. Poggioli, B. Berthon and M. Claret, *FEBS Lett.* **115**, 243, (1980).
40. A. B. Borle and J. Uchikawa, *Endocrinology* **102**, 1725 (1978).
41. A. B. Borle and J. Uchikawa, *Endocrinology* **104**, 122 (1979).
42. E. D. Saggerson and C. A. Carpenter, *FEBS Lett.* **106**, 189 (1979).
43. E. D. Saggerson and C. A. Carpenter, *Biochem. J.* **190**, 283 (1980).
44. D. W. R. MacDonald and E. D. Saggerson, *Biochem. J.* **174**, 641 (1978).
45. H. A. Krebs and H. Henseleit, *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33 (1932).
46. M. W. Slein, in *Methods of Enzymatic Analysis* (Ed. H.-U. Bergmeyer), p. 117. Academic Press, New York (1965).
47. K. Burton, *Biochem. J.* **62**, 315 (1956).
48. T. M. Chan and J. H. Exton, *J. biol. Chem.* **252**, 8645 (1977).
49. D. H. Jenkinson, D. G. Haylett and K. Koller, in *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach* (Ed. R. W. Straub and E. Bolis) p. 89. Raven Press, New York (1978).