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Evidence that Catecholamines Stimulate Renal Gluconeogenesis through an α_1-Type of Adrenoceptor

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1. Noradrenaline stimulates gluconeogenesis through an α-adrenoceptor in renal cortical tubule fragments from fed rats incubated with 5mM-lactate. 2. The selective α_1-adrenoreceptor agonist methoxamine stimulated gluconeogenesis, but the selective α_2-adrenoceptor agonist clonidine was ineffective. 3. The selective α_1-adrenoceptor antagonist thymoxamine blocked the stimulatory effects on gluconeogenesis of noradrenaline and of oxymetazoline (a synthetic α-agonist). The selective α_2-adrenoceptor antagonist yohimbine was ineffective in this respect. 4. It is concluded that noradrenaline and oxymetazoline stimulate gluconeogenesis in rat kidney via an α_1- rather than an α_2-type of adrenoceptor.

Gluconeogenesis in the rat renal cortex can be stimulated by catecholamines (Friedrichs & Schoner, 1973; Klahr *et al.*, 1973; Kurokawa & Massry, 1973; Roobol & Alleyne, 1973; Guder & Rupprecht, 1975; Macdonald & Saggerson, 1977). Studies with selective adrenergic agonists and antagonists have shown that this effect is mediated through an α-type of adrenoceptor (Guder & Rupprecht, 1975; Macdonald & Saggerson, 1977). Recently, a considerable body of pharmacological evidence has accumulated consistent with the hypothesis that there may be more than one type of α-adrenoceptor. This distinction was first made between α-receptors on presynaptic sites and those on postsynaptic sites. Langer (1974) proposed that postsynaptic α-receptors be referred to as α_1 and the presynaptic α-receptors, mediating feedback control by catecholamines of their own secretion from nerve endings, as α_2. Subsequently this concept has been generalized to include the possibility that α_2-receptors are also to be found in other than presynaptic sites; e.g. in platelets (Kafka *et al.*, 1977; Wood *et al.*, 1979; Hoffman *et al.*, 1979), uterus (Hoffman *et al.*, 1979; Wood *et al.*, 1979), submandibular gland (Wood *et al.*, 1979), parotid gland (Wood *et al.*, 1979), melanocytes (Berthelsen & Pettinger, 1977), guinea-pig kidney (Jarrott *et al.*, 1979) and rat kidney cortex (U'Pritchard *et al.*, 1978). It is presumed therefore that the distinction between the α_1- and α_2-receptors must be functional rather than anatomical, although the reason for this division of α-receptors is not understood at present.

The α-adrenoceptor agonist oxymetazoline stimulates gluconeogenesis in rat renal cortical tubules with considerable potency (Macdonald & Saggerson, 1977). This agonist has been categorized as one that is more active at α_2-receptors (Jones & Michell, 1978). In many systems oxymetazoline would appear to be selective towards the α_2-type of receptor (Starke *et al.*, 1975*b*; Berthelsen & Pettinger, 1977; Drew, 1977, 1978; Pichler & Kobinger, 1978; Jarrott *et al.*, 1979), but in some others the experimental evidence suggests selectivity towards α_1-receptors (Struyker-Boudier *et al.*, 1975; Drew, 1976; Doxey, 1979). The actions of oxymetazoline in rat renal tubule fragments differ in several respects from those of adrenaline or noradrenaline. Firstly, oxymetazoline does not stimulate gluconeogenesis in the absence of extracellular Ca^{2+} or in the presence of low concentrations of Ca^{2+}. The natural catecholamines, however, stimulate the process at all tested Ca^{2+} concentrations (Macdonald & Saggerson, 1977; P. Kessar & E. D. Saggerson, unpublished work). Secondly, the stimulation of renal gluconeogenesis by oxymetazoline is less sensitive to abolition by ouabain than that brought about by noradrenaline (Saggerson & Carpenter, 1979). Thirdly, oxymetazoline does not stimulate gluconeogenesis when succinate is the supporting substrate (Macdonald & Saggerson, 1977) whereas noradrenaline does (Guder & Rup-

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precht, 1975). Fourthly, noradrenaline, but not oxymetazoline, can increase the efflux of $^{45}Ca^{2+}$ from tubules prelabelled with this radionuclide (P. Kessar & E. D. Saggerson, unpublished results). In addition, in rat liver, which appears to contain only α_1-type adrenoceptors (Wood *et al.*, 1979; Hoffman *et al.*, 1979), oxymetazoline does not stimulate glycogenolysis or gluconeogenesis (Garrison & Borland, 1979). It was therefore the purpose of this study to investigate the possibility that stimulation of renal gluconeogenesis by α-adrenoceptor agonists might involve α_2-receptors or both α_1- and α_2-receptors, which are reported as being present in the proportions of 4:1 respectively in rat renal cortex (U'Pritchard *et al.*, 1978). A functional α_2-receptor appears to operate in kidney to mediate catecholamine inhibition of renin secretion (Berthelsen & Pettinger, 1977). This effect can also be demonstrated in the isolated rat kidney using the α_2-adrenoceptor agonist clonidine (Vandongen & Greenwood, 1975).

In the present study to investigate the type of α-adrenoceptor(s) involved in the control of gluconeogenesis we have used both the selective agonists methoxamine (α_1-selective, Drew 1976, 1977, 1978) and clonidine (α_2-selective, Starke *et al.*, 1974; Drew, 1977, 1978) and the selective blocking agents thymoxamine (α_1-antagonist: Drew 1976, 1978) and yohimbine (α_2-antagonist: Starke *et al.*, 1975*a*; Doxey *et al.*, 1977; Drew, 1978).

Materials and Methods

Chemicals

These were obtained and treated as described by Macdonald & Saggerson (1977, 1978). In addition, noradrenaline bitartrate was from Sigma and oxymetazoline hydrochloride was a gift from E. Merck, Darmstadt, German Federal Republic. The following were generously provided by Professor D. H. Jenkinson (Department of Pharmacology, University College London): clonidine, methoxamine, thymoxamine and yohimbine.

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, Herts., U.K.) until the time of experimentation when they weighed 160–180g.

Isolation of tubule fragments from renal cortex

This was as described by Macdonald & Saggerson (1977).

Incubation techniques

All procedures were identical to those described by Macdonald & Saggerson (1977). Incubations were for 1h in 4ml volumes containing Krebs–Ringer bicarbonate (Krebs & Henseleit, 1932), 5mM-sodium L-lactate, 1.27mM-Ca^{2+} and 10mg of fatty-acid-poor albumin/ml.

Analytical methods

After incubation, flask contents were deproteinized and glucose was measured as described previously (Macdonald & Saggerson 1977). In all experiments the small amount of glucose initially present in non-incubated preparations was also determined and subtracted from experimental values. Tubule DNA was measured by the method of Burton (1956).

Statistical methods

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

Results and Discussion

Effects of selective α-agonists

Gluconeogenesis was stimulated by the selective α_1-agonist methoxamine. The maximum effect was

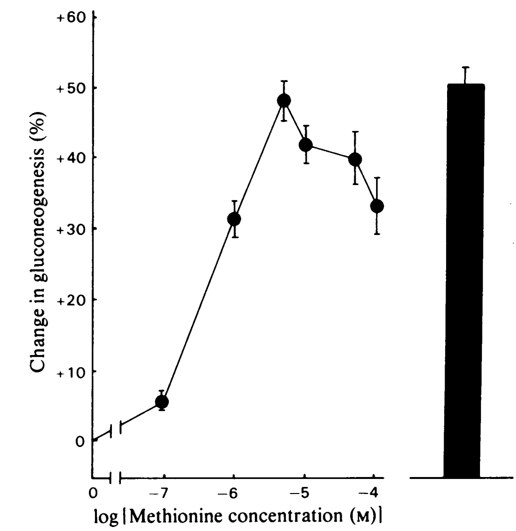

Fig. 1. *Effect of methoxamine on gluconeogenesis*
Tubule fragments were incubated for 1h with 5mM-lactate. The values are means for four separate experiments. The bars indicate S.E.M. All concentrations of methoxamine significantly stimulated glucose formation (P values are: 0.1 μM, <0.02; 1 μM, 5 μM, 10 μM, 50 μM all <0.01; 100 μM, <0.05). The histogram shows the effect of 1 μM-noradrenaline ($P<0.001$). Basal gluconeogenesis was $3.81 \pm 0.33\,\mu$mol/h per mg of DNA. The mean tubule DNA/ml of flask contents was 63 μg.

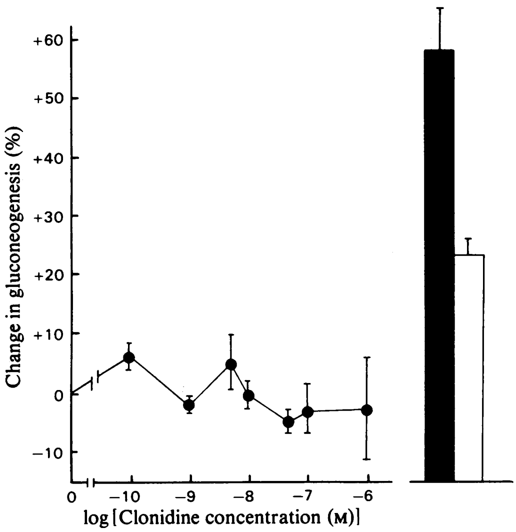

Fig. 2. *Effect of clonidine on gluconeogenesis*
Tubule fragments were incubated for 1 h with 5 mM-lactate. The values are means for four separate experiments. The bars indicate S.E.M. The filled histogram shows the effect of 1 μM-noradrenaline ($P < 0.01$) and the open one the effect of 10 nM-oxymetazoline ($P < 0.01$). Basal gluconeogenesis was 3.54 ± 0.25 μmol/h per mg of DNA. The mean tubule DNA/ml of flask contents was 60 μg.

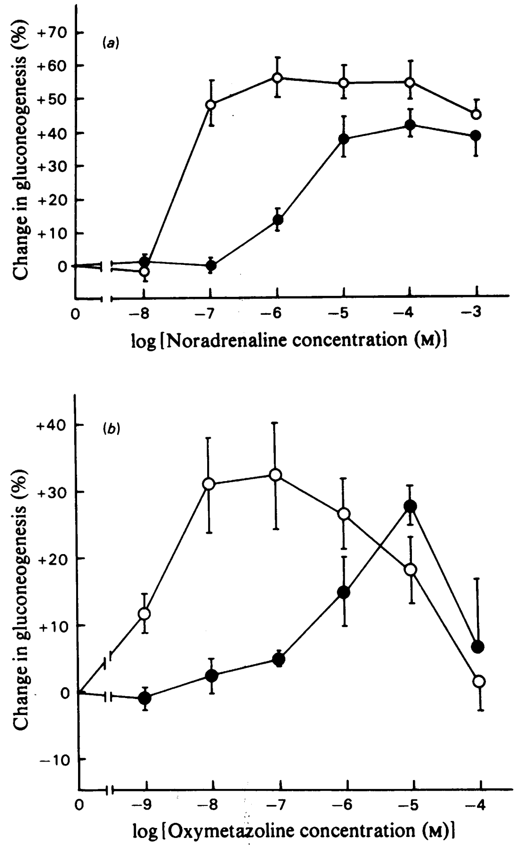

Fig. 3. *Effect of thymoxamine on the stimulation of gluconeogenesis by noradrenaline and oxymetazoline*
Tubule fragments were incubated for 1 h with 5 mM-lactate and the indicated concentrations of (*a*) noradrenaline and (*b*) oxymetazoline with (●) or without (○) 5 μM-thymoxamine. The values are means for four separate experiments in both cases. The bars indicate S.E.M. (*a*) Basal gluconeogenesis with and without thymoxamine was 2.14 ± 0.06 and 2.16 ± 0.10 μmol/h per mg of DNA respectively. The mean tubule DNA/ml of flask contents was 63 μg. (*b*) Basal gluconeogenesis with and without thymoxamine was 2.66 ± 0.16 and 3.07 ± 0.18 μmol/h per mg of DNA respectively. The mean tubule DNA/ml of flask contents was 67 μg.

seen with a drug concentration of 5 μM, which increased gluconeogenesis by approx. 50% (Fig. 1). Noradrenaline, (1 μM) which is the concentration of this agonist that gives maximum stimulation of the process (Guder & Rupprecht, 1975; Saggerson & Carpenter, 1979), also stimulated gluconeogenesis by approx. 50%. On the other hand, gluconeogenesis was not significantly affected ($\leqslant$6%) by any of the tested concentrations (0.1 nM–1 μM) of the selective α_2-agonist clonidine (Fig. 2). That the tubules used in this experiment were normally responsive is shown by the 58% stimulation seen with 1 μM-noradrenaline. These tubule preparations also responded to oxymetazoline (10 nM).

Effects of selective α-adrenoceptor blocking agents

The α-blocker thymoxamine is extremely selective for the postsynaptic α_1-type of adrenoceptor (Drew, 1976, 1977) having little effect at α_2-receptors. Figs. 3(*a*) and 3(*b*) show that 5 μM-thymoxamine caused considerable blockade of the actions of noradrenaline and oxymetazoline, displacing both dose–response curves to the right. Thymoxamine also caused a slight (non-significant) diminution in the maximum response to both noradrenaline and oxymetazoline. In the experiment shown in Fig. 3(*b*) thymoxamine caused a small, but non-significant, decrease in the basal rate of gluconeogenesis but this effect was not always seen (e.g. Fig. 3*a*). As found previously, (Macdonald & Saggerson, 1977) higher concentrations of oxymetazoline had a considerable inhibitory effect on gluconeogenesis.

The α-blocker yohimbine has been found to be suitable as a selective competitive antagonist of α_2-adrenoceptors in the concentration range 3–

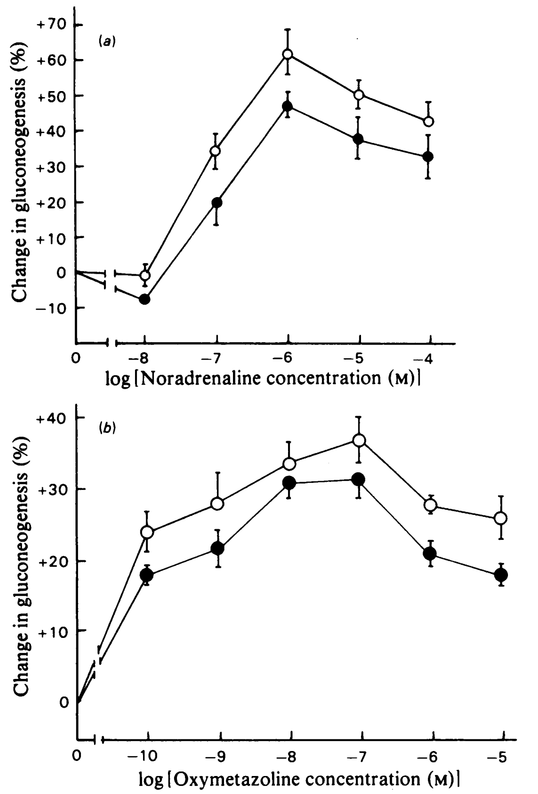

Fig. 4. *Effect of yohimbine on the stimulation of gluconeogenesis by noradrenaline and oxymetazoline*
Tubule fragments were incubated for 1 h with 5 mM-lactate and the indicated concentrations of (*a*) noradrenaline and (*b*) oxymetazoline with (●) or without (○) 0.1 μM-yohimbine. The values are means for four separate experiments in both cases. The bars indicate S.E.M. (*a*) Basal gluconeogenesis with and without yohimbine was 2.51 ± 0.33 and $2.55 \pm 0.27\,\mu$mol/h per mg of DNA respectively. The mean tubule DNA/ml of flask contents was 63 μg. (*b*) Basal gluconeogenesis with and without yohimbine was 3.39 ± 0.20 and $3.36 \pm 0.13\,\mu$mol/h per mg of DNA respectively. The mean tubule DNA/ml of flask contents was 43 μg.

130 nM (Starke *et al.*, 1975*a*; Marshall *et al.*, 1977). Figs. 4(*a*) and 4(*b*) show that 0.1 μM-yohimbine slightly diminished the response to all tested concentrations of noradrenaline and oxymetazoline. There was however little or no shift to the right of these dose–response curves, implying little or no competetive antagonism by this drug. Yohimbine had no effect upon basal gluconeogenesis in either experiment.

General discussion

The combination of the results obtained with the selective agonists and antagonists strongly suggests that α_1-, but not α_2-, adrenoceptors are involved in the α-adrenergic stimulation of renal gluconeogenesis. The site of this process in the kidney is the proximal tubule (Guder & Schmidt, 1974). It is possible therefore that this part of the nephron is devoid of α_2-adrenoceptors and the α_2-agonist binding sites reported by U'Pritchard *et al.* (1978) may be in other structures. Alternatively, the proximal tubular cells might contain α_2-receptors that do not couple to the effector system responsible for the stimulation of the gluconeogenic process.

In the system used here oxymetazoline would appear to act as an α_1-type of adrenoceptor agonist. This is contrary to many, but not all, assignments made for this drug on the basis of work with other systems (see introduction). The actions of this drug are anomalous and not clearly understood.

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References

Berthelsen, S. & Pettinger, W. A. (1977) *Life Sci.* **21**, 595–606
Burton, K. (1956) *Biochem. J.* **62**, 315–323
Doxey, J. C. (1979) *Eur. J. Pharmacol.* **54**, 185–189
Doxey, J. C., Smith, C. F. C. & Walker, J. M. (1977) *Br. J. Pharmacol.* **60**, 91–96
Drew, G. M. (1976) *Eur. J. Pharmacol.* **36**, 313–320
Drew, G. M. (1977) *Eur. J. Pharmacol.* **42**, 123–130
Drew, G. M. (1978) *Br. J. Pharmacol.* **64**, 293–300
Friedrichs, D. & Schoner, W. (1973) *Biochim. Biophys. Acta* **304**, 142–160
Garrison, J. C. & Borland, M. K. (1979) *J. Biol. Chem.* **254**, 1129–1133
Guder, W. G. & Rupprecht, A. (1975) *Eur. J. Biochem.* **52**, 283–290
Guder, W. G. & Schmidt, U. (1974) *Hoppe-Seyler's Z. Physiol. Chem.* **335**, 273–278
Hoffman, B. B., De Lean, A., Wood, C. L., Schocken, D. D. & Lefkowitz, R. J. (1979) *Life Sci.* **24**, 1739–1746
Jarrott, B., Louis, W. J. & Summers, R. J. (1979) *Br. J. Pharmacol.* **65**, 663–670
Jones, L. M. & Michell, R. H. (1978) *Biochem. Soc. Trans.* **6**, 673–688
Kafka, M. S., Tallman, J. F., Smith, C. C. & Costa, J. L. (1977) *Life Sci.* **21**, 1429–1438
Klahr, S., Nawar, T. & Schoolwerth, A. C. (1973) *Biochim. Biophys. Acta* **496**, 448–457
Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
Kurokawa, K. & Massry, S. G. (1973) *J. Clin. Invest.* **52**, 961–964
Langer, S. Z. (1974) *Biochem. Pharmacol.* **23**, 1793–1800
Macdonald, D. W. R. & Saggerson, E. D. (1977) *Biochem. J.* **168**, 33–42

Macdonald, D. W. R. & Saggerson, E. D. (1978) *Biochem. J.* **174**, 641–646

Marshall, I., Nasmyth, P. A., Nicholls, C. G. & Shepperson, N. B. (1977) *Br. J. Pharmacol.* **59**, 510P

Pichler, L. & Kobinger, W. (1978) *Eur. J. Pharmacol.* **52**, 287–295

Roobol, A. & Alleyne, G. A. D. (1973) *Biochem. J.* **134**, 157–165

Saggerson, E. D. & Carpenter, C. A. (1979) *FEBS Lett.* **106**, 189–192

Starke, K., Montel, W., Gayk, W. & Merker, R. (1974) *Naunyn-Schmeideberg's Arch. Pharmacol.* **285**, 133–150

Starke, K., Borowski, E. & Endo, T. (1975*a*) *Eur. J. Pharmacol.* **34**, 385–388

Starke, K., Endo, T. & Taube, H. D. (1975*b*) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **291**, 55–78

Struyker-Boudier, H., De Boer, J., Smeets, G., Lein, E. J. & Van Rossum, J. (1975) *Life Sci.* **17**, 377–386

U'Pritchard, D. C., Charness, M. E., Robertson, D. & Snyder, S. H. (1978) *Eur. J. Pharmacol.* **50**, 87–89

Vandongen, R. & Greenwood, D. M. (1975) *Clin. Exp. Pharmacol. Physiol.* **2**, 583–588

Wood, C. L., Arnett, C. D., Clarke, W. R., Tsai, B. S. & Lefkowitz, R. L. (1979) *Biochem. Pharmacol.* **28**, 1277–1282