Effect of compound D-600 (methoxyverapamil) on gluconeogenesis and on acceleration of the process by α -adrenergic stimuli in rat kidney tubules

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1. Tubule fragments were isolated from renal cortex of fed rats and glucose formation was measured after incubation with 5 mM-sodium lactate. 2. Compound D-600 (10–100 μ M) decreased gluconeogenesis from lactate. This inhibition of the process by compound D-600 increased with increasing extracellular Ca^{2+} concentration, was overridden by noradrenaline and diminished by starvation for 24 h. 3. Inhibition of lactate-supported gluconeogenesis by compound D-600 was not prevented by the α_1 -adrenoceptor antagonist thymoxamine. 4. Compound D-600 had little effect on gluconeogenesis from 2-oxoglutarate and increased gluconeogenesis from succinate. 5. Compound D-600 opposed stimulation of gluconeogenesis by noradrenaline or oxymetazoline (a selective α -adrenoceptor agonist) in a manner suggesting that compound D-600 is an α -adrenoceptor blocker. Oxymetazoline was more sensitive than noradrenaline to blockade by both compound D-600 and by the conventional α -adrenoceptor antagonist phentolamine. Noradrenaline became more sensitive to blockade by compound D-600 when extracellular Ca^{2+} was decreased. 6. Compound D-600 did not block stimulation of gluconeogenesis by angiotensin or cyclic AMP.

Gluconeogenesis in rat renal cortex is stimulated by catecholamine hormones through an α -type of adrenoceptor (Guder & Rupprecht, 1975; Macdonald & Saggerson, 1977). Basal gluconeogenesis in this tissue is stimulated by addition of extracellular Ca^{2+} (Krebs *et al.*, 1963; Rutman *et al.*, 1965; Kurokawa & Rasmussen, 1973; Alleyne *et al.*, 1973) and Ca^{2+} has been assigned the possible role of an intracellular messenger in other systems responding to α -adrenergic stimuli (Selinger *et al.*, 1973; Butcher, 1975; Keppens *et al.*, 1977; Assimacopoulos-Jeannet *et al.*, 1977). Verapamil and its methoxy derivative, compound D-600, are of interest since they inhibit Ca^{2+} -associated activities in various tissues (see review by Singh, 1978). However, there have only been two brief reports dealing with the effects of these agents on gluconeogenesis; one in liver where compound D-600 had little effect on basal or on glucagon- and adrenaline-stimulated gluconeogenesis from lactate (Fain *et al.*, 1975); the other in renal cortex where verapamil and compound D-600 stimulated gluconeogenesis from succinate or 2-oxoglutarate (Gordon & Ferris, 1977). It was therefore of interest to investigate the effect of compound D-600 on the stimulation of renal gluconeogenesis by α -adrenergic agents.

Materials and methods

Chemicals

These were obtained and treated as described by Macdonald & Saggerson (1977, 1978) and Kessar & Saggerson (1980). In addition compound D-600 [α -(3-{2-(3,4-dimethoxyphenyl)ethyl}methylamino)-propyl]-3,4,5-trimethoxy- α -(1-methylethyl)benzene-acetonitrile] was a gift from Professor D. H. Jenkinson (Department of Pharmacology, University College London). [Asp¹, Ile⁵]Angiotensin was a gift from Dr. P. Whitton (Department of Biochemistry, St. George's Hospital Medical School, London).

Animals

These were male Sprague–Dawley rats bred in the animal colony at University College. Fed animals were maintained on GR3EK diet (E. Dixon and Sons, Ware, Herts., U.K.) and weighed 160–180 g at the time of the experiment. Starved rats had the diet removed at approx. 09.30 h on the day before an experiment. At this time they also weighed 160–180 g. All animals had unlimited access to drinking water.

Isolation of tubule fragments from renal cortex

This was as described by Macdonald & Saggerson (1977) in a medium consisting of Krebs–Ringer bicarbonate (Krebs & Henseleit, 1932) containing collagenase (2 mg/ml), fatty-acid-poor albumin (20 mg/ml) and 1.27 mM- Ca^{2+} .

Incubation techniques

Incubations were always commenced immediately after the preparation of tubule fragments and were performed as described by Macdonald & Saggerson (1977). In all cases these were for 1 h and, unless stated otherwise, were in Krebs–Ringer bicarbonate containing 5 mM-sodium L-lactate, 1.27 mM- Ca^{2+} and fatty-acid-poor albumin (10 mg/ml).

Analytical methods

Glucose was measured enzymically in samples from deproteinized incubations as described by Macdonald & Saggerson (1977, 1978). In all experiments the small amount of glucose initially present in non-incubated preparations was also determined and subtracted from experimental values.

Statistical methods

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

Results and discussion

Effect of compound D-600 concentrations on basal gluconeogenesis

Under standard incubation conditions (1.27 mM- Ca^{2+} with lactate as substrate) compound D-600 consistently decreased basal gluconeogenesis. In a total of 15 experiments (pooled from Figs. 1a–1e) percentage decreases were: with 3 μM -compound D-600, $9.0 \pm 0.9\%$; with 10 μM , $16.0 \pm 0.9\%$; with 30 μM , $27.0 \pm 1.0\%$; with 100 μM , $44.6 \pm 0.7\%$ ($P < 0.001$ versus control in every case). When the extracellular Ca^{2+} concentration was decreased to 0.13 mM (Fig. 1b) compound D-600 decreased basal gluconeogenesis to a lesser extent; i.e. with 3 μM -compound D-600, $6.9 \pm 1.4\%$ ($P < 0.02$ versus control); with 10 μM , $8.6 \pm 2.0\%$ ($P < 0.05$ versus control); with 30 μM , $15.3 \pm 1.2\%$ ($P < 0.01$ versus control); with 100 μM , $28.1 \pm 1.6\%$ ($P < 0.001$ versus control). The Ca^{2+} dependence of this inhibitory action of compound D-600 is considered further below (see Fig. 8).

Effect of compound D-600 concentrations on gluconeogenesis stimulated by noradrenaline, oxymetazoline, angiotensin and cyclic AMP

Gluconeogenesis from lactate in this system is maximally stimulated by noradrenaline in the con-

centration range 0.1–1 μM (see Figs. 2 and 4; also see Guder & Rupprecht, 1975; Saggerson & Carpenter, 1979; Kessar & Saggerson, 1980). A $56 \pm 4\%$ stimulation of gluconeogenesis with 1 μM -noradrenaline was observed (Fig. 1a). Compound D-600 (3 μM) slightly increased both the noradrenaline-stimulated absolute rate and the increment in gluconeogenic rate due to noradrenaline. Thereafter higher compound D-600 concentrations (10–100 μM) diminished the percentage effect of noradrenaline and the increment in gluconeogenesis due to the hormone. A significant $23 \pm 2\%$ ($P < 0.001$) residual stimulation by noradrenaline was still apparent at 100 μM -compound D-600. When the extracellular Ca^{2+} concentration was decreased to 0.13 mM (Fig. 1b), not only was the percentage effect of compound D-600 on basal gluconeogenesis decreased (see above) and the absolute rate of gluconeogenesis considerably decreased, but the effectiveness of compound D-600 in blocking the response to 1 μM -noradrenaline was increased. Under these conditions all tested concentrations of compound D-600 diminished the response to noradrenaline, 100 μM -compound D-600 causing essentially complete blockade of the noradrenaline effect.

Stimulation of renal tubular gluconeogenesis by adrenaline or noradrenaline appears to be exerted through an α -type of adrenoceptor (Guder & Rupprecht, 1975; Macdonald & Saggerson, 1977). Oxymetazoline is a synthetic selective α -adrenoceptor agonist that can also stimulate this process with considerable potency (Macdonald & Saggerson, 1977; Kessar & Saggerson, 1980), but differs from the catecholamine in its actions in several respects. First, oxymetazoline does not stimulate gluconeogenesis in the absence of extracellular Ca^{2+} or in the presence of low Ca^{2+} concentrations. Adrenaline and noradrenaline, however, stimulate the process at all tested Ca^{2+} concentrations (Macdonald & Saggerson, 1977; P. Kessar & E. D. Saggerson, unpublished work). Secondly, the stimulation of gluconeogenesis by oxymetazoline is less sensitive to abolition by ouabain than that brought about by noradrenaline (Saggerson & Carpenter, 1979). Thirdly, noradrenaline, but not oxymetazoline, can increase the efflux of $^{45}\text{Ca}^{2+}$ from tubules prelabelled with this radionuclide (P. Kessar & E. D. Saggerson, unpublished work). Gluconeogenesis from lactate in this system is maximally stimulated by oxymetazoline in the concentration range 10 nM–0.1 μM (see Figs. 3 and 5 and also Kessar & Saggerson, 1980). A $36 \pm 5\%$ stimulation of gluconeogenesis with 0.1 μM -oxymetazoline was observed in the absence of compound D-600 with extracellular 1.27 mM- Ca^{2+} (Fig. 1c). Compound D-600 was far more effective in blocking this action of oxymetazoline than it was in opposing the effect of noradrenaline under the same conditions (Fig.

Fig. 1. Effect of compound D-600 on basal gluconeogenesis and on gluconeogenesis stimulated by noradrenaline, oxymetazoline, angiotensin or cyclic AMP

Tubule fragments were prepared and incubated with lactate as described in the Materials and methods section, except that in experiment (b) the extracellular Ca^{2+} was adjusted to 0.13 mM at the start of the incubation. The values are means for four separate experiments in each case. The bars indicate S.E.M. and, where not shown, lie within the symbol. Symbols: O, basal gluconeogenesis; ●, gluconeogenesis in the presence of added agonist; ■, increment in gluconeogenesis due to agonist. (a) The agonist was noradrenaline (1 μM). The mean tubule DNA content was $45 \pm 3 \mu\text{g}$ per ml of flask contents; (b) the agonist was noradrenaline (1 μM); extracellular $[\text{Ca}^{2+}] = 0.13 \text{ mM}$. The mean tubule DNA content was $40 \pm 3 \mu\text{g}$ per ml of flask contents; (c) the agonist was oxymetazoline (0.1 μM). The mean tubule DNA content was $45 \pm 3 \mu\text{g}$ per ml of flask contents; (d) the agonist was angiotensin (0.1 μM). The mean tubule DNA content was $45 \pm 1 \mu\text{g}$ per ml of flask contents; (e) the agonist was cyclic AMP (0.1 mM). The mean tubule DNA content was $47 \pm 2 \mu\text{g}$ per ml of flask contents.

1a). This difference was quite striking in that 30 μM-compound D-600 was sufficient to completely abolish the effect of oxymetazoline. It has been concluded that adrenaline and noradrenaline stimulate renal tubule gluconeogenesis via an α -adrenergic mechanism since 10 μM-phentolamine (an α -adrenoceptor blocker) is able to completely abolish the stimulatory effects seen with these

agonists at 1 μM concentration (Guder & Rupprecht, 1975; Macdonald & Saggerson, 1977). Furthermore, 10 μM-propranolol (a β -adrenoceptor blocker) is totally ineffective in this respect (Guder & Rupprecht, 1975; Macdonald & Saggerson, 1977). Nevertheless, it was thought possible that the lower sensitivity of noradrenaline to blockade by compound D-600 might be attributable to some β -

adrenergic effect that would not be seen with the more selective agonist oxymetazoline. It was eventually concluded that this was unlikely since addition of $10\mu\text{M}$ -propranolol to incubations in an experiment similar to that shown in Fig. 1(a) did not alter the sensitivity of the noradrenaline effect to blockade by compound D-600 (two experiments; results not shown).

A further demonstration of the ability of compound D-600 to diminish the stimulatory effects of α -adrenergic agonists was seen when compound D-600 was tested against $5\mu\text{M}$ -methoxamine, which is a selective α_1 -adrenoceptor agonist. This substance stimulates renal tubular gluconeogenesis from lactate by approx. 50% with an optimum concentration of $5\mu\text{M}$ (Kessar & Saggerson, 1980). In two experiments (results not shown) it was found that $100\mu\text{M}$ -compound D-600 totally blocked the effect of methoxamine.

Although compound D-600 was effective, to various extents, in blocking α -adrenergic stimulation of gluconeogenesis, it was ineffective against the stimulatory actions of angiotensin or cyclic AMP. Fig. 1(d) shows that, in agreement with previous findings (Guder, 1979), angiotensin caused a small but significant stimulation of renal gluconeogenesis. Angiotensin ($0.1\mu\text{M}$), which gives the greatest stimulation in the absence of compound D-600 (see Fig. 6) increased gluconeogenesis by $17.5 \pm 0.5\%$ ($P < 0.001$) (Fig. 1d). Compound D-600 (3 – $100\mu\text{M}$) diminished both basal and angiotensin-stimulated gluconeogenesis, but showed no blockade of the angiotensin effect. Essentially the same result was obtained when compound D-600 was tested against 0.1mM -cyclic AMP (Fig. 1e). This concentration of the cyclic nucleotide has previously been shown to give the optimum effect on renal tubular gluconeogenesis (Guder *et al.*, 1971; Macdonald & Saggerson, 1978).

Effect of compound D-600 on noradrenaline and oxymetazoline dose-response curves

After completion of the above experiments, Blackmore *et al.* (1979) published findings from which it was concluded that verapamil acts as an α -adrenoceptor antagonist in rat hepatocytes. Verapamil was able to block stimulation of glycogenolysis and activation of phosphorylase by adrenaline or phenylephrine as well as inhibiting binding of [^3H]adrenaline to liver plasma membranes (Blackmore *et al.*, 1979). The effectiveness of compound D-600 to alter noradrenaline or oxymetazoline dose-response curves was therefore tested in the renal tubular system. Fig. 2(a) shows that 30 and $100\mu\text{M}$ -compound D-600 shifted noradrenaline dose-response curves to the right. The inhibition of basal gluconeogenesis by compound D-600 was again seen but, surprisingly, this effect was over-

Fig. 2. Effect of compound D-600 on noradrenaline dose-response curves

Tubule fragments were prepared and incubated with lactate as described in the Materials and methods section with the indicated concentrations of noradrenaline. Compound D-600 concentrations are indicated as follows: zero, $\circ$, $30\mu\text{M}$, $\bullet$; $100\mu\text{M}$, $\blacksquare$. The values are means for four separate experiments. The mean tubule DNA content was $45 \pm 2\mu\text{g}$ per ml of flask contents. (a) The values are expressed in absolute terms. The bars indicate s.e.m. and, where not shown, lie within the symbol; (b) the values are normalized by expressing the mean basal value in each case as zero and the maximum value for each curve as 100.

ridden by maximally effective doses of the hormone. The effect of compound D-600 to shift the noradrenaline dose-response curve is better seen in Fig. 2(b), where the data are normalized. Compound D-600 ($30\mu\text{M}$) caused a rightwards shift of the noradrenaline curve of approx. 30-fold and

Fig. 3. Effect of compound D-600 on the oxymetazoline dose-response curve

Tubule fragments were prepared and incubated with lactate as described in the Materials and methods section with the indicated concentrations of oxymetazoline. Compound D-600 was either absent (○) or present at 30 μM (●). The values are means for four separate experiments. The mean tubule DNA content was $43 \pm 2 \mu\text{g}$ per ml of flask contents. (a) The values are expressed in absolute terms. The bars indicated s.e.m.; (b) the values are normalized by expressing the mean basal value in each case as zero and the maximum value for each curve as 100.

100 μM-compound D-600 a shift approx. 200-fold. Figs. 3(a) and 3(b) show a similar experiment performed with oxymetazoline and 30 μM-compound D-600. In this experiment high concentrations of oxymetazoline did not appear to override the inhibition of gluconeogenesis by compound D-600. However, oxymetazoline concentrations in excess of 0.1 μM have an inhibitory effect on gluconeogenesis (see Fig. 5a and also Kessar &

Saggerson, 1980). It is likely that this latter effect limits the extent of the stimulation seen with oxymetazoline when the oxymetazoline dose-response curve is shifted rightwards by some kind of blockade. When these data are normalized (Fig. 3b) it may be seen that the shift of the oxymetazoline dose-response curve on addition of 30 μM-compound D-600 is very considerable, being in excess of three orders of magnitude.

These effects of compound D-600 may be compared with the 8-fold shift in the dose-response curve of phenylephrine stimulation of glycogenolysis seen when rat hepatocytes are treated with 100 μM-verapamil (Blackmore *et al.*, 1979).

Effect of phentolamine on noradrenaline and oxymetazoline dose-response curves

The effects of compound D-600 described above would not be at variance with the possibility that it acts as an α -adrenoceptor antagonist. However, the considerable difference in sensitivity to compound D-600 of the noradrenaline and oxymetazoline effects was puzzling. The effect of a relatively low concentration (1 μM) of the conventional α -adrenoceptor antagonist phentolamine was therefore tested against these two agonists. As expected, phentolamine caused a blockade of the noradrenaline effect on gluconeogenesis (Fig. 4a) resulting in an approx. 60-fold shift of the dose-response curve (Fig. 4b). In the experiment shown in Fig. 5(a) the effect of oxymetazoline was investigated over a wide dose range revealing an effect of low concentrations of this drug that we have seen intermittently but not previously reported. The oxymetazoline dose-response curve appears to consist of two components. Stimulation of the process by 1 nM–1 μM-oxymetazoline with a peak at around 0.1 μM is seen consistently in all our studies. The stimulation by 1–10 pM-oxymetazoline is seen in some, but not all, series of experiments. The reason for this variability is unknown.

Figs. 5(a) and 5(b) show that 1 μM-phentolamine had a profound effect on the response of the system to oxymetazoline. As in the experiments with compound D-600 oxymetazoline was more sensitive than was noradrenaline to the effect of the blocker. If the nature of the stimulation seen with oxymetazoline in the presence of phentolamine relates to the right-hand peak of stimulation seen in the absence of the blocker, the shift in the dose-response curve is again in excess of three orders of magnitude. Greater sensitivity of oxymetazoline to α -adrenoceptor blockade compared with noradrenaline may therefore be a general phenomenon and the findings with compound D-600 need not be regarded as anomalous.

Fig. 4. Effect of phentolamine on the noradrenaline dose-response curve

Tubule fragments were prepared and incubated with lactate as described in the Materials and methods section with the indicated concentrations of noradrenaline. Phentolamine was either absent (○) or present at $1\mu\text{M}$ (●). The values are the means for three separate experiments. The mean tubule DNA content was $40 \pm 1\mu\text{g}$ per ml of flask contents. (a) The values are expressed in absolute terms. The bars indicate S.E.M.; (b) the values are normalized by expressing the mean basal value in each case as zero and the maximum value for each curve as 100.

Fig. 5. Effect of phentolamine on the oxymetazoline dose-response curve

Tubule fragments were prepared and incubated with lactate as described in the Materials and methods section with the indicated concentrations of oxymetazoline. Phentolamine was either absent (○) or present at $1\mu\text{M}$ (●). The values are the means for four separate experiments. The mean tubule DNA content was $37 \pm 3\mu\text{g}$ per ml of flask contents. (a) The values are expressed in absolute terms. The bars indicate S.E.M.; (b) the values are normalized by expressing the mean basal value in each case as zero and the maximum value for each curve as 100.

The effect of compound D-600 on the angiotensin dose-response curve

Fig. 6. shows that $30\mu\text{M}$ -compound D-600 did not cause any significant shift in the angiotensin

dose-response curve. Furthermore, unlike noradrenaline (Fig. 2a), high concentrations of angiotensin did not override the inhibitory effect of compound D-600 on gluconeogenesis. This is further evidence that the blocking action of compound D-600 is selective towards adrenergic events.

Is the inhibitory effect of compound D-600 on lactate-supported gluconeogenesis exerted via an α -adrenoceptor?

Since noradrenaline, but not angiotensin, overrode the inhibitory effect of compound D-600 it seemed possible, though improbable, that compound D-600 might exert this inhibitory effect (in addition to its blockade action) through an α -adrenoceptor. Thymoxamine, which is selective for the α_1 -type of adrenoceptor, blocks stimulation of renal tubular gluconeogenesis by noradrenaline or oxymetazoline (Kessar & Saggerson, 1980). Fig. 7 shows that thymoxamine (1 – $10\ \mu\text{M}$) did not prevent the inhibitory effect of compound D-600. That thymoxamine was active in this experiment was established by testing its action against noradrenaline stimulation of the process. Thymoxamine ($10\ \mu\text{M}$) almost totally blocked the effect of $1\ \mu\text{M}$ -noradrenaline. Hence the inhibitory effect of compound D-600 on the basal rate of gluconeogenesis appears to be distinct from its proposed action as an α -adrenoceptor blocker.

Ca^{2+} dependence of the inhibition of lactate-supported gluconeogenesis by compound D-600

The effect of extracellular Ca^{2+} on gluconeogenesis from lactate was investigated in the presence and absence of $100\ \mu\text{M}$ -compound D-600 (Fig. 8). As reported by many workers (e.g. Krebs *et al.*, 1963; Rutman *et al.*, 1965; Kurokawa & Rasmussen, 1973; Alleyne *et al.*, 1973) the rate of gluconeogenesis was influenced considerably by the extracellular Ca^{2+} concentration. It may be seen in Fig. 8 that, as implied previously (Fig. 1b), inhibition of gluconeogenesis by compound D-600 is also influenced by the Ca^{2+} concentration. As this was increased, both the percentage and the absolute inhibitory effect of compound D-600 was increased. These findings might imply that compound D-600 acts in this respect solely by decreasing the overall effect that Ca^{2+} has on the process. However, this cannot be so since gluconeogenesis from succinate and 2-oxoglutarate, as well as from lactate, is also increased with increasing Ca^{2+} (Kurokawa & Rasmussen, 1973; Alleyne *et al.*, 1973), yet compound D-600 influences gluconeogenesis in a different manner with each of these substrates (see below). Furthermore, Gordon & Ferris (1977) have shown that verapamil has no effect on the gross Ca^{2+} content of renal tubules.

Fig. 6. Effect of compound D-600 on the angiotensin dose-response curve

Tubule fragments were prepared and incubated with lactate as described in the Materials and methods section with the indicated concentrations of angiotensin. Compound D-600 was either absent (O) or present at $30\ \mu\text{M}$ (●). The values are the means for four separate experiments and the bars indicate S.E.M. The mean tubule DNA content was $45 \pm 1\ \mu\text{g}$ per ml of flask contents.

Fig. 7. Effect of thymoxamine on the response of gluconeogenesis to noradrenaline on compound D-600

Tubule fragments were prepared and incubated with lactate as described in the Materials and methods section with the indicated concentrations of thymoxamine. The values are means for four separate experiments. The bars indicate S.E.M. and, where not shown, lie within the symbol. The mean tubule DNA content was $40 \pm 4\ \mu\text{g}$ per ml of flask contents. Symbols: O, no further additions; ●, with $1\ \mu\text{M}$ -noradrenaline; ■, with $10\ \mu\text{M}$ -compound D-600; □, with $30\ \mu\text{M}$ -compound D-600; ▲, with $60\ \mu\text{M}$ -compound D-600; Δ, with $120\ \mu\text{M}$ -compound D-600.

Substrate and nutritional dependence of the effect of compound D-600 on gluconeogenesis

Gordon & Ferris (1977) reported that 100 μM -compound D-600 increased gluconeogenesis from

Fig. 8. Effect of compound D-600 on the response of gluconeogenesis to extracellular Ca^{2+}

Tubule fragments were prepared and incubated with lactate as described in the Materials and methods section, except that the extracellular Ca^{2+} was adjusted to the indicated concentrations at the start of the incubation. The values are means for four separate experiments. The bars indicate S.E.M. and, where not shown, lie within the symbol. The mean tubule DNA content was $40 \pm 1 \mu\text{g}$ per ml of flask contents. Symbols: O, glucose production without compound D-600; ●, glucose production with 100 μM -compound D-600; □, percentage decrease due to compound D-600.

5 mM-succinate or 5 mM-2-oxoglutarate by approx. 50% in tubule fragments isolated from 24-h-starved rats. The experiments shown in Table 1 were therefore performed to ascertain whether the difference in compound D-600 actions between our findings and those of Gordon & Ferris (1977) was attributable to differences in nutritional status or to differences in gluconeogenic substrate. Table 1 shows that, in our hands, the effect of compound D-600 differed considerably with the substrate used. Gluconeogenesis supported by succinate was consistently increased by compound D-600 whereas, as found previously, the process was decreased when lactate was used. When 2-oxoglutarate was the substrate, compound D-600 had little effect, which is at variance with the findings of Gordon & Ferris (1977). Starvation for 24 h diminished the stimulatory and inhibitory effects of compound D-600 seen when succinate and lactate were the substrates respectively.

Conclusion

Compound D-600 has interesting effects in the renal tubular system both directly at the level of the gluconeogenesis process itself and on the α -adrenergic control of the process. Its effects are subtly influenced by Ca^{2+} concentration. Like verapamil in the liver (Blackmore *et al.*, 1979) compound D-600 shows actions characteristic of an α -adrenoceptor blocker. At present though, it is unknown whether compound D-600 achieves its blocking action by interacting with an adrenoceptor or by interacting with a site (or sites) on some effector system normally involved in transmitting signals from the cell surface to the enzymic components of the pathway. It is noteworthy that the effectiveness of compound D-600 as a blocker appears to increase

Table 1. Effect of compound D-600 on gluconeogenesis from lactate, 2-oxoglutarate or succinate in tubule fragments from fed or 24-h-starved rats

Tubule fragments were isolated and incubated for 1 h as described in the Materials and Methods section. The values are means $\pm$ S.E.M. for four experiments in both cases. The values in parentheses indicate the percentage changes due to compound D-600. Statistical significance of effects of compound D-600 is indicated by: * $P < 0.05$; † $P < 0.02$; ‡ $P < 0.001$. The mean tubule DNA contents per ml of flask contents were $48 \pm 4 \mu\text{g}$ and $60 \pm 5 \mu\text{g}$ for the fed and starved states respectively.

Substrate	Compound D-600 (100 μM)	Glucose production ($\mu\text{mol/h}$ per mg of DNA)	
		Fed	Starved (24 h)
Sodium L-lactate (5 mM)	—	2.81 ± 0.14	4.38 ± 0.67
	+	1.59 ± 0.11 ($-43 \pm 2\%$)‡	3.71 ± 0.71 ($-18 \pm 3\%$)†
Sodium 2-oxoglutarate (5 mM)	—	2.40 ± 0.10	3.32 ± 0.64
	+	2.25 ± 0.12 ($-6 \pm 2\%$)*	3.10 ± 0.64 ($-8 \pm 2\%$)*
Sodium succinate (5 mM)	—	2.80 ± 0.14	3.54 ± 0.64
	+	3.74 ± 0.20 ($+33 \pm 2\%$)‡	3.99 ± 0.66 ($+14 \pm 6\%$)

with decreasing Ca^{2+} . It is also unknown at present whether the more direct effects of compound D-600 on gluconeogenesis are at the enzymic level in the pathway. The variation in the effect of compound D-600 with different substrates suggests the involvement of more than one effect of the drug. The ability of noradrenaline to override the inhibitory effect of compound D-600 when lactate is the substrate, and which effect is itself influenced by Ca^{2+} , is particularly interesting.

It is suggested that compound D-600 may be a useful experimental tool in eliciting information about mechanisms involved in information transfer from α -adrenoceptors to components of the gluconeogenic pathway.

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