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THE ALPHA ADRENERGIC RECEPTOR MEDIATED INCREASE IN GUINEA-PIG LIVER GLYCOGENOLYSIS

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Abstract—Amidephrine, a selective α-adrenergic receptor agonist, and isoprenaline, a selective β-receptor agonist, each produced comparable, dose related, increases in the glycogen phosphorylase activity of guinea-pig liver slices, while only isoprenaline produced a statistically significant increase in cyclic AMP levels. These effects were obtained with agonist concentrations which produced similar increases in the rate of glucose release. Although the tissue was only exposed to the drugs for 2 min, it is not thought likely that an earlier peak in cyclic AMP, caused by amidephrine, remained undetected, as the α-agonist did not stimulate adenylate cyclase in a membrane preparation from guinea-pig liver. The effect of amidephrine on phosphorylase activity was abolished by phentolamine at a concentration which did not affect the response to isoprenaline. It is concluded that α-receptor stimulation is as effective as β-receptor stimulation in increasing hepatic glycogenolysis, but that the α-mediated effects do not involve cyclic AMP. The possibility that α effects result from changes in phosphorylase kinase or phosphatase activity brought about by cellular calcium re-distribution is discussed.

Sympathomimetic amines are known to increase glucose release from the liver but there is still much uncertainty about the receptors involved. In many early studies relatively non-selective agonists such as adrenaline and noradrenaline were used, and this made it difficult to interpret some results, either in terms of receptor classification or in terms of the mechanisms leading to increased glycogenolysis. cf. [1–3].

More recently selective agonists have become available and have been increasingly used in such studies. Thus, in guinea-pig liver slices, a selective α-receptor agonist, amidephrine [4, 5], and a selective β-agonist, isoprenaline, each brought about a rise in the rate of glucose release [6]. These observations afforded an opportunity to study the mechanisms mediating the same response elicited by stimulation of each type of adrenergic receptor.

Particular attention has been paid to cyclic AMP, since the relationship of cyclic AMP to the α-receptor is still unclear. Robinson *et al.* [7] have suggested that stimulation of the α-receptor might lead to decreased cyclic AMP levels, and such an effect has been observed [8–10]. In contrast, some evidence indicates that cyclic AMP levels rise [11, 12], and other reports indicate no change in cyclic AMP on stimulation of α-receptors [13, 14]. A preliminary report of some of these findings has appeared [15].

MATERIALS AND METHODS

Liver slices. A male guinea-pig of either the Hartley or the Porton strain was killed by a blow on the head and exsanguinated. The liver was removed as quickly as possible. Liver slices were prepared and incubated for 3 hr in a modified Maclean's medium under conditions very similar to those already described [6, 16]. At the end of the incubation, the slices were transferred to the apparatus shown (Fig. 1). In most experiments slices were incubated in this apparatus for 10 min in 5 ml of medium, and then for three 2-min periods before drugs were added in a fourth 2-min period.

Unless otherwise stated, tissues were treated with

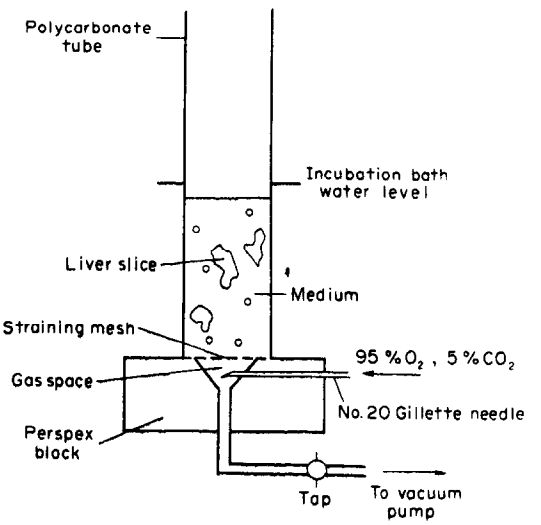

Fig. 1. The 'slice circulator' that was used in most of the experiments. The passage of the gas caused the slices to circulate in the incubation medium and this probably helped to ensure adequate oxygenation. When necessary the medium could be collected through the tap at the base, and up to five slices could be transferred to liquid nitrogen in 5 sec or less. Usually a bank of four circulators with a common Perspex base was used.

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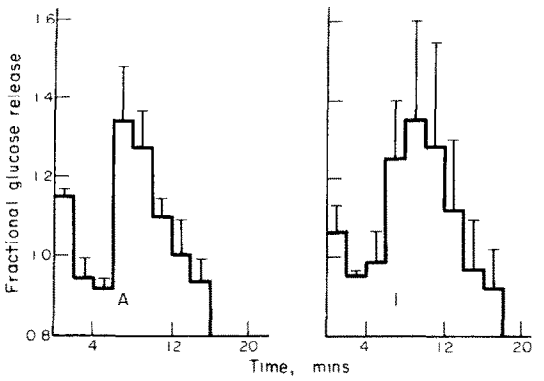

Fig. 2. The time course of increased glucose release from liver slices after the addition of either 10 μM amidephrine (A) or 10 nM isoprenaline (I) for 2 min (horizontal bars). The histograms represent the mean 'fractional glucose release' + one S.E. mean (vertical bars) from four experiments. This measure of glucose release has been used to make some allowance for the considerable variability of the glucose release from guinea-pig liver slices [6, 23]. It is calculated by determining the glucose output from the slices in each time period, and, having obtained the mean value for output in the first three periods, expressing the output in each period as a fraction of this mean.

drugs for 2 min. At this time cyclic AMP levels are probably close to their peak values in liver tissue stimulated by sympathomimetic amines [1], and, additionally, glycogen phosphorylase activation is probably nearly maximal since increased glucose release peaks 2–4 min after drugs have been applied, cf. Fig. 2, [17].

Glucose release. The amount of glucose released by groups of slices (3–5 slices with a wet wt of 80–200 mg) into 5 ml of incubation medium was determined by a slightly modified sub-micro ferricyanide method [6, 18].

Cyclic AMP content of liver slices. At the end of the drug exposure period 3–5 slices were plunged into liquid nitrogen and then transferred to a 1 ml glass homogeniser containing 0.25 ml of incubation medium and 0.5 ml of 5% (w/v) trichloroacetic acid. The homogenate was centrifuged at 5000 *g* for 10 min at 4° and the supernatant then applied to a 3.0×0.5 cm column of Dowex 50×8 (H^+ form). This procedure removed both TCA and unidentified substances which had been found to interfere with some types of protein-binding cyclic AMP assays. Cyclic AMP was eluted with distilled water in the fourth to ninth ml of eluate (inclusive). The eluate was lyophillized and resuspended in 0.5 ml of cyclic AMP assay buffer (46 mM Tris pH 7.4, containing 4 mM EDTA), before the cyclic AMP content of two 100 μl aliquots was determined by a protein binding technique [19].

Adenylate cyclase activity of plasma membrane preparation. A simplified method was developed from an established technique [20]. One chopped whole guinea-pig liver (7–10 g) was placed in 25 ml of 0.25 M sucrose solution containing 1 mM EGTA and 30 mM Tris buffer at pH 7.4, and homogenised first with two 5-sec bursts of a Polytron at the lowest speed setting, and then with eight strokes of a loose fitting Dounce homogeniser. The homogenate was filtered through a double layer of cheesecloth, made up to 400 ml with homogenising medium, and centrifuged at 1000 *g* for 10 min at 4°. The supernatant was then decanted and recentrifuged at 11,000 *g* for a further 10 min at 4°. The 11,000 *g* pellet was resuspended in about 10 ml of homogenising medium and layered over a two step discontinuous sucrose density gradient consisting of a lower 40% (w/v) and an upper 30% (w/v) sucrose solution, containing 30 mM Tris buffer pH 7.4. After centrifugation at 70,000 *g* for 2 hr at 4°, a fraction believed to be mainly plasma membrane was harvested from the 30–40 per cent interface. When a sample of this material was examined under the electron microscope the predominant structures were small sac-like vesicles. There was little contamination with other cell components.

Adenylate cyclase activity in 100 μg (protein wt) of this material was determined in a total volume of 0.2 ml, containing 2 mM ATP, 5 mM $MgCl_2$, 10 mM theophylline, and 30 mM Tris buffer pH 7.4. It was necessary to include an ATP regenerating system made up of 25 μg of creatine kinase and 10 mM phosphocreatine.

Agonists were added immediately before, and antagonists 20 min before, the reaction was initiated by the addition of ATP. After a 20 min incubation at 37° the assay tubes were placed in a boiling water bath for 3 min to terminate the reaction. Samples were diluted and centrifuged at 1300 *g* for 5 min at 4° before the cyclic AMP content was determined by means of a protein binding technique [19].

Glycogen phosphorylase activity of liver slices. A rigorous, established method was suitably modified [21]. Three to four slices were frozen in liquid nitrogen and then homogenised in a 1 ml glass homogeniser containing 0.5 ml of a medium composed of 40 mM β-glycerophosphate buffer pH 6.8, 10 mM NaF and 1 mM EDTA. The homogenate was centrifuged at 10,000 *g* for 10 min at 4°, and the supernatant diluted 1:5 for use in the phosphorylase assay.

Phosphorylase activity was assayed in a vol. of 0.8 ml made up of 0.2 ml of diluted extract and 0.6 ml of glycerophosphate buffer, pH 6.8, containing 1% glycogen (w/v), 75 mM glucose-1-phosphate, 10 mM NaF, and 1 mM EGTA. The glycogen and the enzyme extract were allowed to equilibrate for 10 min before initiation of the reaction by addition of glucose-1-phosphate. After incubation at 37° for 20 min the reaction was terminated by the addition of a 0.2 ml aliquot of the assay mixture to 8 ml of 0.125 M sodium acetate buffer pH 4.0.

In this phosphorylase assay the amount of inorganic phosphate released is related to the enzyme activity.

Phosphate was determined by the method of Lowry and Lopez [22] since this method gave very low blank values. At the end of the phosphorylase assay 0.2 ml of the reaction mixture was added to 8 ml of 0.125 M sodium acetate buffer pH 4.0 to stop the phosphorylase reaction. To this was added 1 ml of a 1% (w/v) solution of ammonium molybdate in 0.15 N sulphuric acid and 1 ml of a 1% (w/v) solution of ascorbic acid. The development of the blue phosphomolybdous colour was enhanced by

Table 1

Isoprenaline			Amidephrine		
Concentration, nm	% increase in glucose release ± one S.E. mean	n	Concentration, μM	% increase in glucose release ± one S.E. mean	n
4	32.4 ± 11.0	6	4	27.4 ± 5.9	6
20	56.6 ± 17.9	7	20	71.3 ± 14.7	7
100	55.4 ± 15.0	6	100	76.6 ± 25.8	5
500	59.6 ± 21.9	6	500	96.5 ± 30.0	6

Dose–response relationships of isoprenaline and amidephrine on glucose release. In these experiments pairs of slices were exposed to drugs for 8 min and glucose release was calculated by Haylett and Jenkinson's method [6]. This was done so that the results could be compared with those obtained by these authors. The results were obtained in two series of experiments. In one series isoprenaline was used, and in the other amidephrine, since only enough slices could be obtained from one animal to construct a dose–response curve for one of the agonists.

warming the tubes in a water bath at 37° for 10 min. The tubes were then removed from the water bath and, after a further 10 min, the optical density was determined at 695 nm. Since there was a slow rise in the optical density of tubes containing tissue extract (cf. [22]), it was difficult to determine the absolute amount of phosphate produced. However, since this increase in any one tube was proportional to the increase in any other, it was possible to express the amount of phosphate produced in the drug treated tubes, and thus the phosphorylase activity, as a percentage of the phosphate produced in the control tubes. The phosphate assay incorporated a blank to correct for any non-enzymic production of phosphate from glucose-1-phosphate, as well as a tissue blank for each tube to correct for any phosphate or other interfering tissue component present in each tissue extract. Further details of the phosphorylase and phosphate assays can be found elsewhere [23].

Protein. Protein was determined by an auto-analytical modification of an established method [24], devised by Technicon Ltd., Chertsey, Surrey. All samples were dissolved in 0.1 N NaOH before analysis.

Materials. Laboratory reagents (Analar, whenever possible) were purchased from BDH, reagents for the cyclic AMP assay in kit form from the Radiochemical Centre, Amersham, U.K., and all biochemicals were from Sigma. Gifts of (−) propranolol HCl, isoprenaline (as (−) isoprenaline bitartrate) and amidephrine (as (±) amidephrine mesylate) were from ICI, Ward Blenkinsop, and Mead Johnson respectively. They are gratefully acknowledged. Phentolamine mesylate was obtained from Ciba.

RESULTS

Effects of amidephrine and isoprenaline on liver slice cyclic AMP levels. Concentrations of amidephrine and isoprenaline were chosen which produced comparable, large, but submaximal, increases in glucose release. This choice of dose minimised the possibility that effects on cyclic AMP levels would be unrelated to glucose effects, which might have been the case had concentrations of the agonists been used which were supramaximal to the glucose release. Other results suggested that in guinea-pig liver slices 20 μM amidephrine and 20 nM isoprenaline would produce comparable effects on glucose release [6], but it was not clear from this data whether these concentrations were sub- or supramaximal. Accordingly, glucose dose–response relationships were determined (Table 1). Although the results were variable—owing at least in part to the inherent variability in the basal rate of glucose release from the slices [23], it seemed that 20 μM amidephrine and 20 nM isoprenaline would be suitable doses. Lower concentrations were not used since it was known that the effects of sympathomimetic amines on hepatic cyclic AMP levels are small [1].

While amidephrine and isoprenaline produced comparable increases in glucose release only isoprenaline significantly elevated cyclic AMP levels (Fig. 3). These results were extended to show that while isoprenaline significantly elevated cyclic AMP levels at all concentrations between 4 nM and 1 μM, amidephrine only raised cyclic AMP levels at 0.1 mM, a concentration at which this drug has weak partial β-agonist activity (Fig. 4) [5]. It is noteworthy that the effect of 0.1 mM amidephrine is only marginally greater than that of 4 nM isoprenaline, and that there is a considerable disparity in the

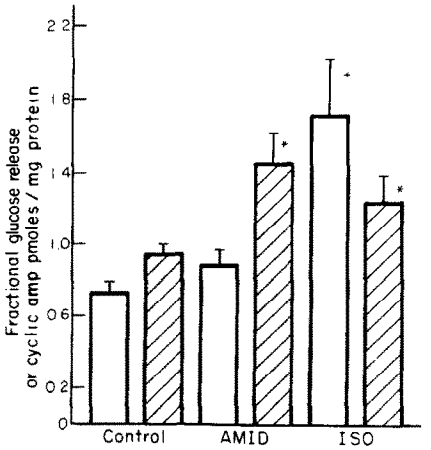

Fig. 3. Effect of 20 μM amidephrine (AMID) and 20 nM isoprenaline (ISO) on glucose release from (□) and cyclic AMP levels in (▨) groups of slices exposed to the agonists for 2 min. Vertical bars represent one S.E. mean. $n = 8$. * = significantly greater than the control at the 5 per cent level. Isoprenaline increases both cyclic AMP and glucose release to rise, whereas amidephrine causes a rise in glucose release but no change in cyclic AMP levels.

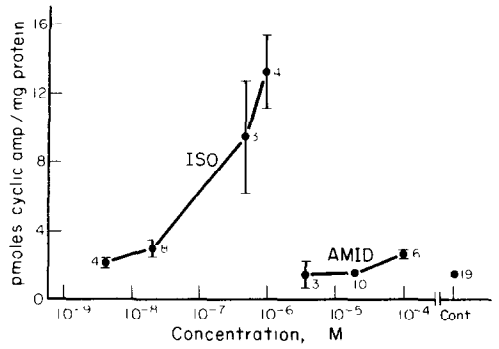

Fig. 4. Dose–response relationships for isoprenaline (ISO) and amidephrine (AMID) on cyclic AMP levels in guinea-pig liver slices. Vertical bars represent two S.E. about the mean where this exceeds the size of the symbol. CONT = control level of cyclic AMP. The figures by the symbols represent the number of observations. While isoprenaline raises the level of cyclic AMP (at the 5 per cent level of significance) amidephrine does not raise this level, except at 10^{-4} M when a small rise occurs (significant at the 5 per cent level), due perhaps to the weak partial β-agonist property of amidephrine (see Text).

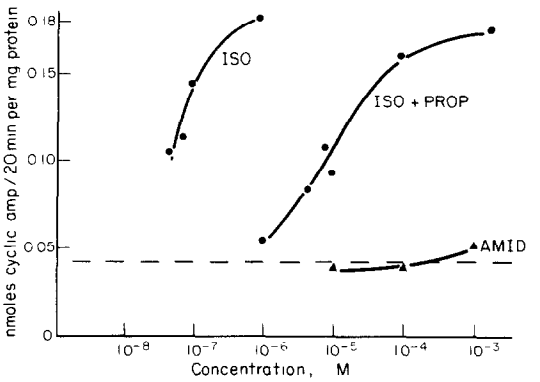

Fig. 5. The effect of isoprenaline and amidephrine on the adenylate cyclase activity of the 11,000 *g* pellet obtained from whole guinea-pig liver. ----- = control activity. ISO = isoprenaline, AMID = amidephrine, PROP = propranolol (1 μM). The points represent a single estimate of enzyme activity. Isoprenaline stimulates adenylate cyclase while amidephrine does not. Propranolol inhibits the response to isoprenaline in a fashion consistent with a competitive inhibition, an indication that the isoprenaline effect is β-mediated.

effects of these concentrations of the agonists on glucose release.

These results suggest that amidephrine increases glucose release by a mechanism which does not involve cyclic AMP, and imply that amidephrine does not stimulate adenylate cyclase. However, the results do not eliminate the possibility that amidephrine might stimulate the cyclase and cause a rise in cyclic AMP which peaks before 2 min have elapsed, after which cyclic AMP levels return to baseline values. To test this possibility the effect of amidephrine on adenylate cyclase activity was determined.

Effects of amidephrine and isoprenaline on adenylate cyclase activity. Sodium fluoride, glucagon, and isoprenaline all stimulated the adenylate cyclase activity present in the most highly purified plasma membrane preparation, i.e. that derived from the 30–40 per cent sucrose density gradient. In contrast, amidephrine did not appreciably stimulate this preparation (Table 2).

Since the response to isoprenaline was variable, and since loss of hormone responsiveness occurs when cyclase preparations are purified, the results were confirmed by testing the effects of the agonists on the cyclase activity of a less purified preparation, namely the 11,000 *g* pellet (see Methods). A typical result from the three preparations tested is shown in Fig. 5. The results confirmed that isoprenaline did and amidephrine did not stimulate adenylate cyclase, and showed that propranolol inhibited isoprenaline-induced stimulation of the enzyme.

Effects of amidephrine and isoprenaline on phosphorylase activity. A number of reports have shown that α-receptor stimulation enhances hepatic gluconeogenesis [25, 26] and it was thus necessary to confirm that α-mediated glucose release was glycogenolytic, and, since amidephrine and isoprenaline

Table 2

Drug	Concentration	Enzyme activity (*n*mole cyclic AMP/ 20 min/mg protein)	*n*
Control	—	0.23 ± 0.01	5
NaF	10.0 mM	1.35 ± 0.36*	2
Glucagon	1.0 μM	0.91 ± 0.07*	2
Amidephrine	10.0 μM	0.24 ± 0.02	4
,,	0.1 mM	0.27 ± 0.03	4
,,	1.0 mM	0.26 ± 0.03	4
Isoprenaline	20.0 nM	0.28 ± 0.05	2
,,	50.0 nM	0.45 ± 0.22*	2
,,	80.0 nM	0.46 ± 0.14*	3
,,	0.2 μM	0.80 ± 0.05*	2
,,	1 μM	0.84 ± 0.04*	4

The effect of various compounds on the adenylate cyclase activity in the guinea-pig liver plasma membrane preparation obtained on the sucrose density gradient. The preparation is stimulated by NaF, glucagon and isoprenaline although the isoprenaline effect is rather variable at low concentrations. In contrast, amidephrine has no appreciable effect. Enzyme activity is shown as the mean ± one S.E. mean or, when $n = 2$, as the mean ± ½ the range.

* Significantly different from the control at the 5 per cent level.

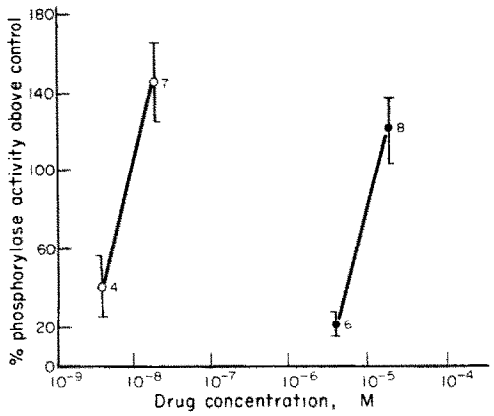

Fig. 6. Dose–response relationships of isoprenaline and amidephrine on phosphorylase activity. (○—○), isoprenaline, (●——●), amidephrine. Vertical bars represent two S.E. mean and the figures by the symbols the number of observations. Amidephrine and isoprenaline have comparable effects on glycogen phosphorylase activity.

had comparable effects on glucose release, to establish that amidephrine caused as great an activation of glycogen phosphorylase as did isoprenaline.

Both amidephrine and isoprenaline stimulated glycogen phosphorylase activity in a dose-related fashion (Fig. 6). Two experiments were carried out to determine the effects of the α-antagonist phentolamine on the phosphorylase response to amidephrine and isoprenaline. 40 μM phentolamine abolished the stimulation of phosphorylase by amidephrine, the percentage phosphorylase activity being 112.5 ± 12.7 per cent above the control with amidephrine alone, and −9.5 ± 3.0 per cent in the presence of phentolamine. In contrast 40 μM phentolamine had no effect on the isoprenaline response the percentage enzyme activities being 100.8 ± 4.8 per cent, above the control with isoprenaline alone and 96.2 ± 17.5 per cent in the presence of phentolamine. These results suggest that amidephrine stimulates phosphorylase by a mechanism which is mediated by the α-adrenergic receptor.

DISCUSSION

It is clear from the current study [14, 27] that cyclic AMP plays no role in the mediation of α-agonist induced increases in hepatic glycogenolysis. However, whereas this and other studies [16, 17], suggest that α- and β-receptor induced hepatic glycogenolysis are processes of comparable magnitude, recent results with rat hepatocytes [27], indicate that the α-mediated process contributes more to adrenaline induced glycogenolysis than the β-stimulated pathway does—a particularly interesting finding since previous work with perfused rat liver identified the hepatic glycogenolytic receptor as a β-receptor [2].

It is probable that at least some of the differences between the various groups of results are due to differences between species, but it is conceivable that some differences arise from the different types of preparation used. In this study, and others [6, 15, 17], liver slices were used, in [27] hepatocytes, and in [1] perfused liver. In each type of preparation the structural integrity of the liver is maintained to different degrees and this could affect the action of various agonists and antagonists, reducing the effect of some and increasing that of others. This may be the case with hepatocytes since the preparative technique involves partial digestion of the tissue with enzymes which could, conceivably, disrupt the character of the receptors which are presumably located on the hepatocyte surface. Even if this process does not occur it is possible that the separation of the liver cells causes the 'exposure' of receptor-like agonist binding sites, which are normally 'hidden'. Thus, the apparent predominance of the α-receptor in isolated hepatocyte glycogenolysis could be a consequence of the preparation used.

To settle this point more detailed pharmacological information, such as dose ratio or pA_2 values, must be obtained so that the receptors may be classified by techniques which are independent of the preparation used. Until such information is available any evaluation of the relative importance of α- and β-receptors must be somewhat speculative.

The current study clearly shows that amidephrine can stimulate phosphorylase as much as isoprenaline can, and that the mechanism of action of the two agonists is different since they have different effects on cyclic AMP levels. However, the study does not elucidate the pathway by which amidephrine increases phophorylase activity. In rabbit and guinea-pig liver slices [17] amidephrine produces a marked increase in ^{45}Ca efflux, whereas isoprenaline, even at concentrations far higher than those required to produce substantial glucose release, has little effect on ^{45}Ca efflux. It is probable that this increased efflux is a result of a redistribution of intracellular calcium stores, and, since calcium can enhance the glycogen phosphorylase-phosphorylase-b-kinase interaction [28] and/or inhibit phosphorylase phosphatase [29] it is possible that amidephrine induced calcium fluxes indirectly regulate hepatic phosphorylase by some effect on the phosphatase and/or the kinase.

Results from rabbit liver suggest that increases in hepatic phosphorylase activity following splanchnic nerve stimulation may be due to changes in phosphorylase phosphatase activity [30]. It would be most interesting if it could be shown that the effects of nerve stimulation were inhibited by α-antagonists. Indeed, if the response to splanchnic nerve stimulation is α-mediated, in view of results obtained with perfused liver [2], the interesting possibility arises that neuronally induced glycogenolysis is α-mediated and humourally induced glycogenolysis β-mediated. Then, the physiological importance of the two receptors and their different methods of phosphorylase activation would depend largely on the circulating level of adrenaline and on the adrenergic innervation of the liver; two factors which would vary both interspecifically and with the physiological state of the animal.

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