Lack of desensitization against α-agonists and vasopressin in the liver

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A heterologous and homologous desensitizaton of the glycogenolytic response against 3',5'-cAMP independent hormones in isolated hepatocytes has been reported [Biochem. J. (1981) 200, 509–514]. We re-examined this phenomenon in isolated perfused rat livers, isolated hepatocytes during stationary incubation and in isolated perifused hepatocytes. The release of glucose and the activation of glycogen phosphorylase were followed in the presence of phenylephrine (10^{-5} M) or vasopressin (2.5×10^{-8} M). A desensitization against these hormones could not be observed in the presence of exogenous calcium (1.3 mM). When calcium-free media were applied, the perfused liver became successively resistant toward the action of phenylephrine (or vasopressin), but regained sensitivity immediately after addition of 1.3 mM calcium to the medium. It is concluded that in isolated hepatocytes desensitization against hormones acting via mobilization of intracellular calcium is an artifact resulting from the experimental conditions.

Lack of desensitization *Calcium* *α-Agonists* *Vasopressin* *Hepatocyte (Perfused rat liver)*

1. INTRODUCTION

The term desensitization is used to describe a successive refractoriness of target cells toward a hormonal agonist after a single or repetitive hormonal stimulation. This desensitization may be hormone specific (homologous) or non-specific (heterologous) when exposure of cells to a given hormone results in a diminished response to other hormones. Desensitization phenomena have been mainly reported for hormones which act via generation of 3',5'-cAMP (for review see [1]).

Recently a desensitization against α-adrenergic agonists has been observed in isolated rat hepatocytes which was homologous as well as heterologous against the vasoactive peptides vasopressin and angiotensin II [2,3]. Since these hormones are thought to act mainly via a mobilisation of cellular Ca^{2+} [4–6], it was concluded that this type of desensitization might have resulted from a depletion or an unavailability of a common calcium pool [2]. Such a depletion caused by a first stimulus would lead to a refractoriness to a subsequent stimulus. It will be shown here, that this desensitization is restricted to conditions where calcium-depleted hepatocytes are used. Under more physiological conditions desensitization against the α-agonist phenylephrine or against vasopressin is no longer observed. It is concluded that the α-agonist-mediated desensitization described in [2] is an artifact resulting from the experimental conditions. Part of this work has been presented elsewhere [7].

2. MATERIALS AND METHODS

Fed male Wistar rats (180–250 g) (Zentralinstitut für Versuchstierzucht, Hannover-Linden) were used in all experiments. Isolation and perfusion of livers were performed as described earlier [8], except that an open perfusion system was used. The perfusate consisted of Krebs-Ringer bicarbonate (KRB) buffer with half the amount of $CaCl_2$ (1.3 mM) present: it was gassed with car-

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bogen (95% O_2, 5% CO_2) at a temperature of 34°C. Unless otherwise mentioned all additions were made to the reservoir. The flow was adjusted to 5 $ml.g^{-1}.min^{-1}$. The perfusate was directly used for enzymatic determination of glucose as in [9]. Hepatocytes were isolated as described elsewhere [10]. For the stationary incubation cells (80–100 mg wet wt/ml) suspended in KRB buffer containing 2 g/100 ml bovine serum albumin (BSA) were incubated at 34°C under continuous gassing with carbogen in a shaking water bath (80 strokes/min). At times indicated in the figures, aliquots (0.1 ml) of the cell suspension were withdrawn, deproteinized by addition of 1 ml of 3.5% (w/v) perchloric acid and neutralized with 2 N KOH.

Perifusion of hepatocytes was performed as in [11], using cells equivalent to 100 mg wet wt/chamber and a flow of 0.8 ml/min of KRB buffer at 34°C. The perifusate was collected and directly used for glucose determinations.

For the determination of phosphorylase activity in perfused livers, a small aliquot (0.5–1 g) of liver tissue was removed and immediately frozen in liquid nitrogen. The frozen tissue was homogenized after addition of 3 parts (w/v) of a medium containing 0.3 M NaF, 20 mM EDTA and 100 mM imidazole–HCl (pH 6.0) using an Ultraturrax homogenizer for 30 s. After centrifugation for 2 min at 18000 × g, glycogen phosphorylase a activity was measured at 25°C in the supernatants as in [12]. When isolated hepatocytes were the source of phosphorylase, an aliquot of 0.5 ml of cell suspension was added to 2 ml of the above medium and homogenized for 5 s at 0°C. Oxygen was measured polarographically in the liver outflow using a Clark type electrode connected to a Gilson oxygraph. The oxygen content of the perfusate in the reservoir was determined as in [13]. Under the conditions used, KRB buffer contained 1.62 ± 0.03 μg atom $O.ml^{-1}$ (n = 5).

Biochemicals and enzymes were obtained from Boehringer Mannheim; phenylephrine, propranolol and vasopressin came from Sigma (Munich); glucagon was purchased from Serva (Frankfurt). All other chemicals were of analytical grade and came from Merck (Darmstadt). Phentolamine was a generous gift from CIBA-GEIGY GmbH (Wehr/Baden).

3. RESULTS

In contrast to the report in [2] where isolated hepatocytes were used, a desensitization following repetitive stimulation with phenylephrine or vasopressin could not be detected in experiments with isolated perfused livers. As depicted in fig.1, the second stimulation was unimpaired, in spite of the fact that for the first stimulation a maximal effective dose of phenylephrine (10^{-5} M) had been applied. The effect of glucagon on glucose release was likewise unimpaired (not shown) which is in agreement with [2].

The efficacy of a second dose of phenylephrine on glycogenolysis in the isolated perfused rat liver as compared to the first one is summarized in table 1. There is no significant difference in the glycogenolytic response between successive additions of the α-agonist. Vasopressin (10^{-9} M) as the second stimulus elicited likewise an immediate response (fig.1).

The authors in [2], who used isolated hepatocytes in stationary incubation, concluded from the transient nature of activation of glycogen phosphorylase under continued exposure to an α-agonist or vasopressin, that this was indicative of an increasing refractoriness of the cells to the respective hormone (and other hormones). This

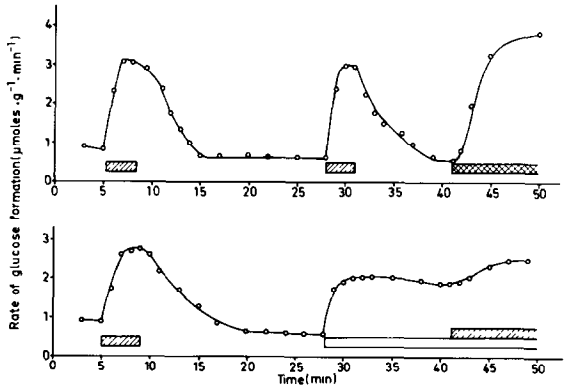

Fig.1. Lack of desensitization against the hormonal action of phenylephrine and vasopressin in the isolated perfused rat liver. Open liver perfusion was carried out as given in section 2. The medium contained the hormones for the time periods indicated by the different bars. The concentration of phenylephrine (hatched bars) was 10^{-5} M, that of vasopressin (open bars) 10^{-9} M, and that of glucagon (cross-hatched bars) 10^{-7} M.

Table 1

Effect of repetitive administration of phenylephrine on glucose production by isolated perfused rat liver

	Rate of glucose formation (μmol.g^{-1}.min^{-1})	
	First administration of 10^{-5} M phenylephrine	Second
Control	0.89 ± 0.05	0.94 ± 0.06
Phenylephrine	3.29 ± 0.15	3.30 ± 0.21

Livers were perfused with KRB buffer in an open system as indicated in the legend to fig.1. The numbers represent the results from 6 expts (means ± SEM) performed as demonstrated in fig.1. The rates of glucose formation before or between successive administrations of phenylephrine are given as control values, those in the presence of phenylephrine represent the peak values

conclusion was based on their finding that phosphorylase could no longer be reactivated by a second administration of the hormone. In our experiments using isolated hepatocytes in stationary incubation not only the first, but also a second addition of phenylephrine was followed by a transient activation of phosphorylase of about the same degree (fig.2). Both transients were accompanied by increased rates of glucose release (fig.2).

The fact that a single application of phenylephrine led only to a transient activation of phosphorylase (fig.2) cannot be interpreted as increasing refractoriness toward the hormone, but results most likely from the degradation of phenylephrine by the hepatocytes. This is in line with the observation that the second bolus of phenylephrine is almost immediately followed by a similar transient activation of phosphorylase (fig.2). When isolated livers were continuously perfused with a medium containing 10^{-5} M phenylephrine, the effect on glucose release remained constant over more than 30 min (fig.3A). The presence of the β-antagonist propranolol, added to suppress residual β-activity, had no significant effect on the stimulation of the glucose release by phenylephrine, whereas the α-blocking agent phentolamine abolished this effect almost completely (fig.3B). Similarly, a persistent effect on glucose release was observed when the liver was

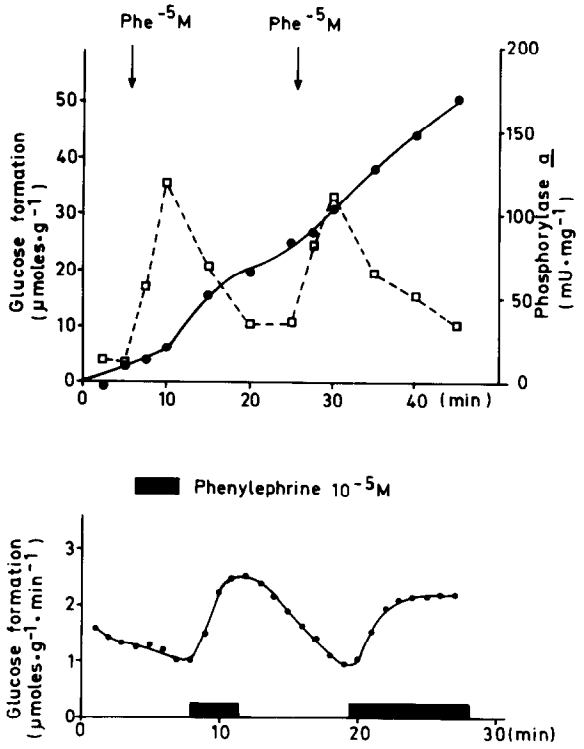

Fig.2. Effect of repetitive administration of phenylephrine on glycogenolysis in isolated hepatocytes in stationary incubation or in perifusion. For the stationary incubation (upper panel) hepatocytes (120 mg wet wt/ml) were incubated at 34°C under continuous gassing with carbogen in a 100 ml plastic beaker in a shaking water bath (80 strokes/min). Phenylephrine was added at the indicated time points (arrows) to give a final concentration of 10^{-5} M. For the determination of glucose (●—●) or phosphorylase activity (□---□) aliquots were taken from the incubation mixture at the time points indicated. Further conditions are given in section 2. For the perifusion experiments (lower panel) 100 mg wet cells were placed in the perifusion chamber. The perifusate consisted of KRB buffer. The flow was adjusted to 0.8 ml/min. The presence of phenylephrine (10^{-5} M) in the perifusate is indicated by the horizontal bars.

perfused in the presence of vasopressin (1–25 nM) (fig.1, lower panel; fig.3A). This supports the view that the transient action of phenylephrine added to the reservoir reflects degradation of phenylephrine rather than desensitization.

To avoid the disadvantages of a stationary incubation we have performed similar experiments with perifused hepatocytes (fig.2, lower panel;

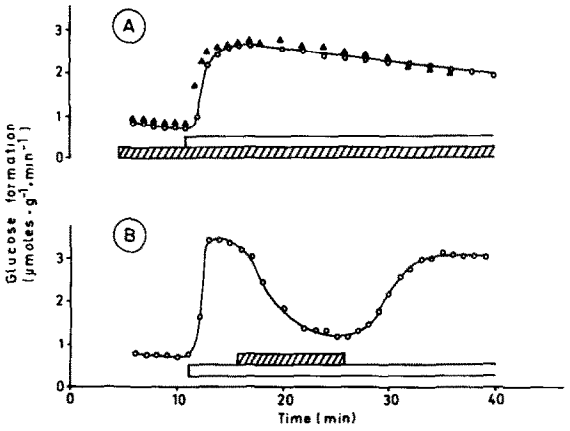

Fig.3. Effect of continuous infusion of phenylephrine or vasopressin on glycogenolysis in the isolated perfused liver. Experimental conditions were as given in the legend to fig.1. The concentration of phenylephrine was 10^{-5} M (○), that of vasopressin $2.5.10^{-8}$ M (▲), respectively (open bars). Where indicated by hatched bars, the perfusion medium contained propranolol (10^{-6} M) (fig.3A), or phentolamine (10^{-5} M) (fig.3B).

table 2). This model compares well with the isolated perfused liver as indicated by the phenylephrine-stimulated oxygen consumption and a similar glucose/oxygen ratio (table 2). With perifused isolated hepatocytes again a desensitization did not occur as indicated by the unimpaired response of glucose production following a second administration of the α-agonist (fig.2, lower panel).

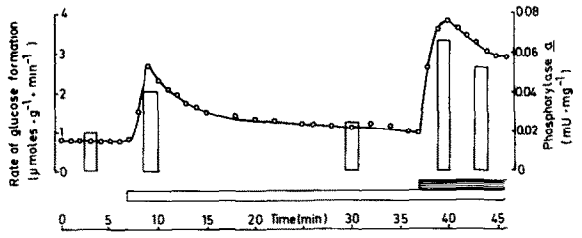

Fig.4. Apparent refractoriness against the glycogenolytic action of phenylephrine during Ca^{2+}-free liver perfusion and resensitization by addition of calcium to the medium. Liver perfusion was carried out as in fig.1, except that a Ca^{2+}-free KRB buffer was used as the perfusate. Phosphorylase *a* activity (open columns) and glucose production rates (○—○) were determined at the time points indicated. The presence of phenylephrine (10^{-5} M) in the perfusion medium is indicated by the open horizontal bar. The hatched horizontal bar indicates the presence of 1.3 M $CaCl_2$ in the medium.

When isolated livers were perfused with a calcium-'free' medium, a steady decline in hormonal response was observed during a continuous infusion of hormone (fig.4). Basal values of glucose production were reached within 20–30 min after start of the phenylephrine infusion. An increase in dosage of phenylephrine or the addition of vasopressin at this time were without effect. Under the same conditions the glycogenolytic effect of glucagon remained unchanged (not shown).

The response to phenylephrine however, was restored immediately after addition of $CaCl_2$ to a

Table 2

Comparison between perifused hepatocytes and isolated perfused livers of the effects of phenylephrine on glucose production and oxygen consumption

	Increment in rate of		
	Glucose formation (μmol.g^{-1}.min^{-1})	Oxygen consumption (μg atom[O].g^{-1}.min^{-1})	Glucose–O ratio
Perifused hepatocytes (n = 4)	1.06 ± 0.17	0.55 ± 0.05	1.9 ± 0.4
Isolated perfused liver (n = 5)	2.44 ± 0.12	1.39 ± 0.11	1.8 ± 0.2

Basal rates of oxygen consumption were 2.52 and 2.96 μg atom (O).g^{-1}.min^{-1}, those of glucose production 0.93 and 0.88 μmol.g^{-1}.min^{-1} for perifused cells and isolated perfused livers, respectively (means ± SEM). The concentration of phenylephrine was 10^{-5} M. The measurements were performed when the phenylephrine-induced increase in oxygen consumption had reached its peak value (usually 2–4 min after addition of phenylephrine). For further experimental conditions see section 2

final concentration of 1.3 mM (fig.4), whereas addition of $CaCl_2$ alone in the absence of phenylephrine resulted only in a small transient increase of glucose release, the rate of which was less than 10% of that seen in the presence of hormone (not shown). This indicates that the intracellular calcium stores necessary for hormonal response will be exhausted during continuous stimulation with phenylephrine unless there is access to calcium from the medium. Again the changes in phosphorylase *a* activity paralleled those in glucose production (fig.4). A similar pattern was observed when vasopressin instead of phenylephrine was used (not shown).

4. DISCUSSION

We conclude that in the isolated perfused liver an apparent desensitization against hormones acting via mobilization of endogenous calcium, only occurs when the cellular calcium stores have been exhausted. The lack of desensitization against phenylephrine cannot be explained by a residual β-effect of this catecholamine as it persists also in the presence of the β-antagonist propranolol. Moreover, vasopressin, which binds most likely only to one class of receptors, likewise exhibits no desensitization in the isolated perfused liver. To demonstrate desensitization in isolated hepatocytes fairly unphysiological concentrations of this hormone have been used, i.e., 230 nM by [3]. As the liver in vivo is continuously perfused with a medium containing more than 1 mM calcium, a degree of calcium depletion which might lead to desensitization will never occur.

In order to explain the results in [2] we have to assume that their isolated hepatocytes had lost a considerable part of their mobilizable calcium during or after the cell preparation. Such a loss of calcium during liver cell preparation has been discussed in detail [14]. Unfortunately the authors in [2] do not report on the calcium content of their cell population. The calcium content of the isolated hepatocytes used in our study was comparable to that of rat liver (0.99 ± 0.04 ($n = 9$) *vs.* 0.93 ± 0.14 ($n = 7$) μmol.g wet wt^{-1}) and in these preparations we obtained the same results as seen with the intact perfused liver, the latter considered to be the 'reference tissue' for most studies with isolated hepatocytes. It is concluded that heterologous (and homologous) desensitization against phenylephrine and vasopressin is an artifact.

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