Effects of Vasopressin, Angiotensin II and Phenylephrine on Hepatic Ketogenesis and Fatty Acid Synthesis

Takahide NOMURA, Masakatsu TACHIBANA, Hiroshi MAEKAWA, Hiroko NOMURA, Ken IZUHARA and Yasumichi HAGINO
Department of Pharmacology, Fujita-Gakuen Health University School of Medicine, Kutsukake-cho, Toyoake, Aichi 470-11, Japan

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Abstract—Studies were conducted to clarify the effects of vasopressin, angiotensin II and phenylephrine on hepatic ketogenesis and fatty acid synthesis. Hepatocytes from fed rats were incubated with oleate or octanoate. Vasopressin stimulated fatty acid synthesis as well as lactate and pyruvate accumulation in the presence of oleate. In accordance with this action, vasopressin caused a marked decrease in ketogenesis from oleate. When octanoate was added as a substrate, vasopressin failed to inhibit ketogenesis. Neither angiotensin II nor phenylephrine affected ketogenesis or fatty acid synthesis. The results in the present study show that there are vasopressin-mediated reciprocal changes in ketogenesis from oleate and fatty acid synthesis in isolated hepatocytes.

Vasopressin, angiotensin II and α-adrenergic agonists are known to exert similar metabolic effects in the liver via a Ca²⁺-linked cyclic AMP-independent mechanism. The most well-established of these effects is stimulation of glycogenolysis and activation of glycogen phosphorylase (1, 2). However, there is a controversy as to whether or not these hormones also have similar actions on hepatic ketogenesis, glycolysis and fatty acid synthesis. Ketogenesis has been shown to be inhibited by vasopressin when oleate is added as a substrate (3, 4). On the other hand, α-adrenergic agonists have been reported to stimulate ketone body formation from palmitate (5). Regarding fatty acid synthesis, vasopressin and angiotensin II have been reported to stimulate (6), to inhibit (7) or to have no effect (3). The effects of the three hormones on glycolysis are also poorly defined. Vasopressin has been reported to increase glycolytic flux (3, 6) or to exert no effect on lactate release (2), whereas slightly decreased output of lactate and pyruvate by catecholamines has been documented (8).

It is well established that there is a regulatory link between ketogenesis and fatty acid synthesis in the liver (9–11). For example, increased ketogenesis by glucagon correlates with a decreased rate of fatty acid synthesis and concomitant reduction of [malonyl-CoA], a potent inhibitor of entry of long-chain fatty acyl-CoA into mitochondria (12, 13). In contrast, insulin decreases ketogenesis, producing an increase in fatty acid synthesis (14). The present study was therefore designed to compare the effects of vasopressin, angiotensin II and phenylephrine on ketogenesis, glycolysis and fatty acid synthesis in isolated rat hepatocytes and to gain further insight into the hormonal regulation of ketogenesis.

Materials and Methods

Animals: Male Wistar rats (300–400 g) were subjected to a 12 hr-light/12 hr-dark cycle, the light period starting at 7:30 a.m. The rats were allowed free access to water and standard laboratory food (Oriental Yeast Co., Tokyo, Japan).

Isolation and incubation of hepatocytes and assays of metabolites: Preparation of hepatocytes was commenced between 9:00 a.m. and 10:00 a.m. by the method of Berry and Friend (15) with the modifications
described by Harris (16). The glycogen content of the hepatocytes was 278±15 (N=9, mean±S.E.M.) μmol of glucose/g wet wt. The cells were suspended (35–45 mg wet wt.) in 2 ml of Krebs-Henseleit buffer (pH 7.4) supplemented with 2.5% (w/v) bovine serum albumin (essentially fatty acid free and dialyzed, Fraction V, Sigma Chemical Co.) under an atmosphere of 95% O2/5% CO2 (v/v) in stoppered 25 ml Erlenmeyer flasks. The concentration of added fatty acid was 1 mM for oleate (long-chain fatty acid) and 0.5 or 1 mM for octanoate (medium-chain fatty acid). Incubations were conducted in a shaking water bath at 37°C for 30 min. Hormones were added at zero time. In a preliminary study, we measured glucose release at 10 min, 20 min and 30 min. It was found that the rate of glucose release in the presence of a hormone exceeded that of the control in all three 10 min periods (results not shown). Thus, metabolic actions of three hormones are thought to be maintained during 30 min incubation. Metabolites were measured spectrophotometrically by enzymatic methods in KOH-neutralized HClO4 extracts of cell suspensions. Glucose was determined according to Slein (17), pyruvate and lactate according to Hohorst et al. (18), acetoacetate and β-hydroxybutyrate according to Williamson et al. (19), and glycogen as described by Harris (16). Ketogenesis was expressed in terms of an accumulation of total ketone bodies (acetoacetate plus β-hydroxybutyrate). Oxidation of fatty acids was assayed by the accumulation of acid-soluble radioactive products (mainly ketone bodies) and 14CO2 from [1-14C]oleate (20). The extent of [1-14C]oleate esterification was determined as described by McCune et al. (21). Results are reported in terms of the sum of the total μmoles of [1-14C]oleate incorporated into mono-, di-, and triacylglycerol plus phospholipids. The rate of fatty acid synthesis was estimated by 3H2O (0.5 mCi/ml) incorporation as described by Harris (16).

Statistical analysis: The results are expressed as means±S.E.M. Statistical evaluation of the data was made by means of Student’s t-test for paired data.

Sources of materials: Chemicals were of the highest grade commercially available. Collagenase (Type II) was obtained from Worthington Biochemical Corp. [Arginine]-vasopressin and angiotensin II (human) were purchased from Peptide Institute, Inc. (Minoh, Japan). L-Phenylephrine hydrochloride was from Sigma. Nucleotides, coenzymes and crystalline enzymes were obtained from Boehringer Mannheim (W. Germany) or from Sigma. Radioactive compounds and scintillation solution were obtained from New England Nuclear.

**Results**

Dosage response of vasopressin, angiotensin II and phenylephrine on glucose release, ketogenesis and glycolysis in the presence of oleate: All three hormones increased glucose release dose-dependently in the presence of oleate (Fig. 1). As determined graphically, vasopressin was not only the most potent agonist but its efficacy was also significantly higher than the other two hormones.

Increasing concentrations of vasopressin caused a progressive inhibition of ketogenesis from oleate (Fig. 2). The maximal effect was seen at 10^-8 M and was equivalent to 30% inhibition. Endogenous ketogenesis (i.e., ketogenesis in the absence of exogenous oleate) was not affected by vasopressin (results not shown). Vasopressin caused a marked accumulation of lactate and pyruvate in the presence of oleate (Fig. 2). The maximal effect was also seen at 10^-8 M. The data presented in Fig. 1 and Fig. 2 clearly indicate that the dose-response of vasopressin on ketogenesis correlated very closely with those on lactate and pyruvate accumulation and glucose release, the concentration for half-maximal effect on all three parameters being approximately 3×10^-10 M.

Lactate and pyruvate accumulation was significantly increased by a higher concentration of angiotensin II (10^-4 M) (Table 1), although the effect was less potent than vasopressin (Fig 2). Phenylephrine did not affect lactate and pyruvate accumulation. Neither angiotensin II nor phenylephrine affected ketogenesis from oleate (Table 1).
Fig. 1. Effects of vasopressin, angiotensin II and phenylephrine on glucose release in the presence of oleate. Incubations were conducted for 30 min. Each point represents the mean±S.E.M. of at least four hepatocyte preparations. Symbols: ○, vasopressin; □, angiotensin II; △, phenylephrine. *P<0.05, compared with corresponding control values.

Fig. 2. Effect of vasopressin on lactate and pyruvate accumulation and ketogenesis in the presence of oleate. Incubations were conducted for 30 min to determine the effect of vasopressin on lactate and pyruvate accumulation (○) and acetoacetate and β-hydroxybutyrate accumulation (●) in the presence of oleate. Each point represents the mean±S.E.M. of five or six hepatocyte preparations. *P<0.05, compared with corresponding control values.
Table 1. Effects of angiotensin II and phenylephrine on lactate and pyruvate accumulation and keto genesis in the presence of oleate

| Additions       | Lactate plus pyruvate (µmol/30 min/g wet wt.) | Total ketone bodies (µmol/30 min/g wet wt.) |
|-----------------|---------------------------------------------|--------------------------------------------|
| Angiotensin II  |                                             |                                            |
| 0               | 2.0±0.8                                     | 20.6±2.8                                   |
| 10−6 M          | 1.6±0.8                                     | 19.4±2.5                                   |
| 10−5 M          | 2.6±0.9                                     | 18.8±2.4                                   |
| 10−4 M          | 4.5±1.2*                                    | 19.7±2.2                                   |
| Phenylephrine   |                                             |                                            |
| 0               | 2.7±0.6                                     | 22.7±3.0                                   |
| 10−5 M          | 3.4±0.7                                     | 20.3±2.7                                   |
| 10−4 M          | 2.9±0.5                                     | 20.8±3.0                                   |

* Incubations were conducted for 30 min. 1 mM oleate was present in all incubations. Results are expressed as the mean±S.E.M. for four to five hepatocyte preparations from fed rats. Values which are significantly different from control incubations are indicated by *. P<0.05.

Table 2. Effects of hormones on [1-14C]oleate esterification and the oxidation of [1-14C]oleate to 14CO2 and radioactive acid-soluble products

| Additions       | Esterification (µmol/30 min/g wet wt.) | 14CO2 formation (µmol/30 min/g wet wt.) | Acid-soluble products (µmol/30 min/g wet wt.) |
|-----------------|----------------------------------------|----------------------------------------|----------------------------------------|
| [1-14C]Oleate (1 mM) | 3.00±0.28                             | 1.27±0.13                            | 4.86±0.63                             |
| [1-14C]Oleate—Vasopressin (10−7 M) | 3.07±0.32                             | 1.67±0.15*                           | 3.82±0.38*                           |
| [1-14C]Oleate—Angiotensin II (10−6 M) | 2.98±0.28                             | 1.54±0.16*                           | 4.47±0.67                             |
| [1-14C]Oleate—Phenylephrine (10−5 M) | 2.90±0.24                             | 1.54±0.16*                           | 4.65±0.74                             |

* Incubations were conducted for 30 min. 1 mM [1-14C]oleate was present in all incubations. Results are expressed as µmol oleate utilized per 30 min per g wet wt. (the mean±S.E.M. of four hepatocyte preparations). Values which are significantly different from control incubations are indicated by *. P<0.05.

[1-14C]oleate: Vasopressin (10−7 M) decreased the formation of radioactive acid-soluble products from [1-14C]oleate by 21% (Table 2). Neither angiotensin II nor phenylephrine affected it. These observations are in good agreement with the effects of hormones on ketogenesis (Fig. 2 and Table 1). None of the three hormones affected oleate esterification, whereas production of 14CO2 from [1-14C]oleate was significantly increased by all three hormones (Table 2).

Effects of hormones on glycogen utilization and fatty acid synthesis in the presence of oleate: All three hormones markedly accelerated glycogen utilization (Table 3). Glycogen has been reported to be one of the major substrates for fatty acid synthesis (22). Therefore, stimulatory effects of hormones on fatty acid synthesis would be a logical consequence of their action on glycogen utilization. Indeed, vasopressin (10−7 M) stimulated fatty acid synthesis by 49% (Table 3). However, neither angiotensin II nor phenylephrine affected fatty acid synthesis.

Effects of vasopressin on octanoate-
Table 3. Effects of vasopressin, angiotensin II and phenylephrine on glycogenolysis and fatty acid synthesis

| Additions                  | Glycogen breakdown (µmol/30 min/g wet wt.) | Fatty acid synthesis (µmol/30 min/g wet wt.) |
|----------------------------|--------------------------------------------|----------------------------------------------|
| Oleate (1 mM)              | 44.7±2.1                                   | 0.86±0.16                                   |
| Oleate + Vasopressin (10⁻⁷ M) | 68.9±3.9*                                 | 1.28±0.24*                                 |
| Oleate + Angiotensin II (10⁻⁵ M) | 55.9±3.3*                                | 0.84±0.14                                  |
| Oleate + Phenylephrine (10⁻⁶ M) | 57.1±2.9*                                | 0.85±0.15                                  |

* Incubations were conducted for 30 min. Oleate (1 mM) was present in all incubations. Results are expressed as the mean±S.E.M. for four to five hepatocyte preparations from fed rats. Values which are significantly different from control incubations are indicated by *: P<0.05.

Table 4. Effects of vasopressin on fatty acids-induced metabolic changes in hepatocytes

| Additions                  | Glucose release (µmol/30 min/g wet wt.) | Lactate plus pyruvate | Total ketone bodies | β-Hydroxybutyrate/ Acetoacetate ratio |
|----------------------------|-----------------------------------------|-----------------------|---------------------|-------------------------------------|
| none                       | 32.7±3.9                                | 23.2±1.6              | 2.6±0.1             | 0.18±0.01                           |
| Oleate (1mM)               | 39.3±3.9*                               | 3.2±0.9*              | 20.2±1.8*           | 1.01±0.09*                          |
| Oleate (1mM) + Vasopressin (10⁻⁷M) | 59.3±4.9*                               | 8.7±0.9*              | 15.6±1.7*           | 0.89±0.08*                          |
| Octanoate (0.5mM)          | 36.9±4.0*                               | 6.1±1.7*              | 23.1±1.0*           | 0.74±0.07*                          |
| Octanoate (0.5mM) + Vasopressin (10⁻⁷M) | 55.8±4.5*                               | 12.6±2.0*             | 22.1±1.0            | 0.82±0.09                           |
| Octanoate (1mM)            | 39.6±4.4*                               | 3.1±0.7*              | 38.8±2.6*           | 1.55±0.13*                          |
| Octanoate (1mM) + Vasopressin (10⁻⁷M) | 59.2±5.6*                               | 5.9±0.7*              | 39.0±2.4            | 1.69±0.14                           |

* Incubations were conducted for 30 min. Results are expressed as the mean±S.E.M. for six hepatocyte preparations from fed rats. †P<0.05, compared with incubations without fatty acids (i.e., none).

Discussions

Induced metabolic changes in isolated hepatocytes: Octanoate, unlike a long-chain fatty acid such as oleate, is known to be transported in a carnitine-independent manner into mitochondria (9). Addition of octanoate has been shown to cause a significant increase in glucose release, a striking stimulation of ketogenesis and a marked inhibition of lactate and pyruvate accumulation in hepatocytes from meal-fed rats (23). The metabolic changes induced by octanoate were confirmed in the present study and found to be essentially the same as by oleate (Table 4). Vasopressin caused a significant stimulation of glucose release and lactate and pyruvate accumulation in the presence of 0.5 or 1 mM octanoate. Nevertheless, it failed to affect ketogenesis from either concentration of octanoate (Table 4). The ratio of β-hydroxybutyrate/acetoacetate, an index of the NAD⁺ redox state in the mitosol, was markedly increased both by oleate and octanoate, indicating an active fatty acid oxidation. The ratio in the presence of oleate was significantly decreased by vasopressin, while that in the presence of octanoate was not affected by vasopressin (Table 4).

Discussion

In the present study, we observed that
vasopressin markedly inhibits ketogenesis from oleate. This result is in agreement with the report by Williamson et al. (3). Furthermore, we demonstrated that vasopressin inhibits the formation of acid-soluble products from [1-14C]oleate. Two other hormones, angiotensin II and phenylephrine, were found to have no effect on ketogenesis or the formation of acid-soluble products. Kosugi et al. (5) reported that phenylephrine stimulated ketogenesis from palmitate in isolated hepatocytes from fed rats. The discrepancy between our result and that of Kosugi et al. (5) is probably due to the fact that they used a lower concentration (0.3 mM) of fatty acid and/or added 100 mg/dl glucose to the incubation medium. The reason for the discrepancy needs to be further investigated.

It is clearly shown in this paper that the anti-ketogenic action of vasopressin was promoted in accordance with the progressive accumulation of lactate and pyruvate (Fig. 2), which have an antiketogenic action (24). Phenylephrine and angiotensin II did not produce a stimulation of lactate and pyruvate accumulation. Hue et al. (25) reported that vasopressin and phenylephrine caused an accumulation of fructose 2,6-bisphosphate, which is a potent stimulator of glycolysis. In the present study, however, the marked increase of lactate and pyruvate accumulation was produced only by vasopressin. Furthermore, the effect of vasopressin was observed in the presence of oleate (Fig. 2) but not in the absence of it [control (N=4): 21.3±2.4, plus vasopressin (10^-7 M) (N=4): 23.3±2.6 μmol/30 min/g wet wt.]. Oleate has been shown to inhibit glycolysis (Table 4 and see Ref. 26). Therefore, it is suggested that vasopressin tends to antagonize the inhibition of glycolysis by oleate, while two other hormones, angiotensin II and phenylephrine, fail to antagonize it.

Another important point to stress in this paper is that ketogenesis and fatty acid synthesis were found to be reciprocally controlled by vasopressin when oleate was the substrate. Previous studies have established that glucagon and insulin coordinately control ketogenesis and fatty acid synthesis (13, 14). Nevertheless, so far, there has been no report demonstrating vasopressin-mediated reciprocal changes in ketogenesis and fatty acid synthesis in isolated hepatocytes. We have found that vasopressin stimulates fatty acid synthesis (Table 3) and suppresses ketogenesis from oleate (Fig. 2).

Stimulation of fatty acid synthesis by vasopressin might be brought about by the increase of lactate accumulation. Several studies have documented that lactate inhibits fatty acid synthesis, but also as a hormone-like substance with respect to fatty acid synthesis in the liver (27, 28); i.e., it not only increases the rate of fatty acid synthesis but also enhances the activity of acetyl-CoA carboxylase, the rate-limiting enzyme of fatty acid synthesis. In this study, stimulation of lactate and pyruvate accumulation by vasopressin was found to occur in the presence of oleate but not in the absence of it (discussed above). Furthermore, we observed that fatty acid synthesis was not increased by vasopressin in the absence of oleate [control (N=4): 2.48±0.31, plus vasopressin (10^-7 M) (N=4): 2.66±0.34 μmol/30 min/g wet wt.]. Therefore, it is reasoned that the stimulatory effect of vasopressin on hepatic fatty acid synthesis in the presence of oleate is mainly attributed to the stimulation of lactate and pyruvate accumulation by this hormone.

It has been proposed that the decreased ketogenesis from oleate in response to vasopressin may be related to increased oxidation of oleate through the tricarboxylic acid cycle (4). Indeed, vasopressin was shown to increase 14CO2 formation from [1-14C]oleate (Table 2). However, it was demonstrated in this paper that angiotensin II and phenylephrine, which had no effect upon ketogenesis, also produced a stimulation of 14CO2 formation from [1-14C]oleate (Table 2). Therefore, it is unlikely that the increased oxidation of oleate to CO2 plays a major role in the antiketogenic action of vasopressin. It is clear from the present investigation that vasopressin inhibits ketogenesis from oleate but not from octanoate (Table 4). It is known that octanoate, unlike long-chain fatty acids, is transported in a carnitine-independent
manner into mitochondria and oxidized by β-oxidation to acetyl-CoA (9). The lack of the effect of vasopressin on ketogenesis from octanoate indicates that vasopressin regulates ketogenesis from long-chain fatty acids mainly at sites before β-oxidation. Possible site(s) will be the esterification of long-chain fatty acids and/or the entry of long-chain fatty acyl-CoA into mitochondria (29). In the present study, vasopressin was found to have no effect upon oleate esterification (Table 2). On the other hand, vasopressin was shown to increase fatty acid synthesis in the presence of oleate (Table 3). It is known that malonyl-CoA, which is an intermediate product of fatty acid synthesis, is an inhibitor of entry of long-chain fatty acyl-CoA into mitochondria (13). However, it remains to be seen whether vasopressin increases malonyl-CoA levels under the present condition. Recently, Harano et al. (30) reported that glucagon produced a direct activation of carnitine palmitoyl-transferase through phosphorylation. The possibility of a direct regulation of this enzyme by vasopressin also needs to be investigated.

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