Differential Effects of Sodium Acetoacetate and Acetoacetic Acid Infusions on Alanine and Glutamine Metabolism in Man

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ABSTRACT It has been suggested that ketone bodies might participate in the nitrogen-sparing process occurring during prolonged starvation by inhibiting the muscular production of alanine and glutamine, which are the main gluconeogenic amino acids. The results of the ketone infusion studies on which this theory is based have been reevaluated in this study by following the plasma levels of ketone bodies, alanine, glutamine, and other substrates during 11.5 h in five groups of normal overnight-fasted subjects. Subjects of groups I, II, and III were infused for 3 h, respectively, with Na acetoacetate, Na bicarbonate, or free acetoacetic acid administered in comparable amounts (about 20 μmol/kg per min), whereas group IV was infused with hydrochloric acid (7.0 μmol/kg per min). A control group (V) received no infusion. Na acetoacetate induced a rise in blood pH (+0.1±0.003) and a fall in the plasma levels of alanine (−41.8±4.6%) and glutamine (−10.6±1.4%), whereas free acetoacetic acid had a barely detectable lowering effect on blood pH and induced a rise in alanine (+22.5±8.0%) and glutamine (+14.6±3.2%) levels. Both infusions were associated with a lowering of plasma glucose, which therefore seems independent of the changes in alanine and glutamine concentrations. Sodium bicarbonate reproduced the alkalinizing effect and the hypoalaninemic action of Na acetoacetate, which seems thus unrelated to hyperketonemia. On the other hand, acidification of blood with hydrochloric acid did not mimic the effects of acetoacetic acid.

If the hyperalaninemic and hyperglutaminemic effects of ketone bodies infused in their physiological form (free acids) reflect a stimulation of the muscular output of these amino acids, the participation of ketone bodies in the nitrogen-sparing process of prolonged fasting seems very unlikely. On the other hand, during brief starvation, when both ketogenesis and gluconeogenesis are markedly stimulated, ketone bodies might indirectly contribute in supplying the liver and the kidney with gluconeogenic substrates.

INTRODUCTION

A few years ago, Sherwin et al. (1) suggested that the reduction in alanine (ALA)[1] levels and the remarkable protein conservation observed during prolonged fasting could be related to hyperketonemia. They showed, indeed, that the infusion of sodium β-hydroxybutyrate (βOHB) in postabsorptive or fasted subjects reduces the plasma concentration of ALA, the most important glucogenic amino acid (2). Furthermore, they demonstrated in obese subjects fasted for several weeks, that the hypoalaninemic effect of prolonged Na βOHB infusion was associated with a decrease in the excretion of urea, ammonia, and total nitrogen in urine. Plasma glutamine (GLN) concentration was not measured in these studies, but since it represents the main precursor for renal ammonia production (3), these authors hypothesized that ketone bodies might also reduce the concentration of this amino acid, which plays a key role in renal gluconeogenesis (4). These experiments led to the suggestion that during prolonged starvation, ketone bodies serve not only as a substrate replacing glucose for the brain, but also as a signal to muscle, resulting in a diminution of ALA and GLN production and consequently a reduction in gluconeogenesis from proteins, this process favoring nitrogen sparing. However, some metabolic aspects of fasting cannot easily

This work was presented in part at the 10th Congress of the International Diabetes Federation, Vienna, Austria, September 1979.

Received for publication 19 October 1979 and in revised form 8 April 1980.

[1] *Abbreviations used in this paper*: ALA, alanine; AcAc, acetoacetate; βOHB, β-hydroxybutyrate; FFA, free fatty acid; GLN, glutamine.

be accounted for by this theory. For instance, during brief starvation (a few days), muscular ALA release is increased (5) despite an already marked hyperketonemia. On the other hand, it is difficult to conceive that the inhibitory effect of ketones on muscular ALA release and nitrogen excretion would be most prominent after prolonged fasting, since at that time uptake of ketones by muscle is markedly reduced (6, 7) as compared with early starvation. Recent studies by Yang et al. (8) on obese patients submitted to an hypocaloric diet also tend to refute the hypothesis of a protein-sparing effect of ketone bodies: these authors noticed that the negative nitrogen balance was not improved when a ketogenic rather than a nonketogenic diet was provided to the patients receiving an identical protein and caloric supply.

Because of these considerations, we reassessed in the present work the influence of infusions of ketone bodies on ALA levels and extended our analysis to changes in GLN concentration. Our data confirm the hypoalaninemic effect of ketone bodies infused as their sodium salt. They demonstrate that this effect can be entirely reproduced when sodium acetoacetate (AcAc) is replaced by equimolar amounts of sodium bicarbonate inducing the same degree of blood alkalinization. When acetoacetate was delivered as free acid, a rise rather than a fall in ALA and GLN concentrations was observed. This effect was independent of the acidification process, in that it could not be reproduced with infusions of hydrochloric acid.

METHODS

Subjects and procedures. The studies were performed on postabsorptive healthy subjects of both sexes, aged 18–47 yr (Table I). All were medical students or belonged to the medical staff of the hospital. Candidates were informed of the nature, purpose, and possible risks of the study before consenting. After an overnight fast of 12–15 h, a plastic cannula was placed in an antecubital vein of each arm, one for blood sampling and the other for delivering infusions. The subjects were divided into five groups. After a basal period of 90 min, the subjects of the first four groups were infused at the rate of 3.1 ml/min for 3 h with either Na AcAc (21.5±0.9 μmol/kg per min, group I, $n = 13$), Na bicarbonate (21.0±1.0 μmol/kg per min, group II, $n = 8$), AcAc acid (20.2 ±0.8 μmol/kg per min, group III, $n = 8$), or hydrochloric acid (7.0±0.2 μmol/kg per min, group IV, $n = 7$). At the start of the infusions, a priming dose representing 40 times the amount infused per min was delivered to the subjects at a rate of 6.2 ml/min. Group V ($n = 7$) included control subjects receiving no infusion. The solutions of AcAc acid or hydrochloric acid administered to groups III and IV were, because of their low pH (2.6 and 0.84, respectively), delivered by means of a catheter inserted under local anesthesia in an antecubital vein and advanced to the superior vena cava under fluoroscopic guidance.

Blood was sampled every 30 min during the basal period and during the following 5 h. An additional sample was obtained 10 h after the onset of the infusion. For group IV only, the study was terminated at the end of the 3 h infusion period. No untoward effect was observed during or after these experiments.

Analysis. Standard enzymatic methods were used to determine plasma concentrations of AcAc (9), βOHB (9), glucose (10), lactate (11), GLN (12), and ALA (13). All determinations were performed after prior deproteination of plasma with perchloric acid as described (14), except for ALA, which is currently determined in our laboratory without prior deproteination.[2] Free fatty acids (FFA) were determined according to Trout et al. (15). Plasma immunoreactive insulin was assayed using a Sephadex anti-insulin complex (Phadebas insulin test, Uppsala, Sweden).

Blood gas analysis (pH, P_{CO_2}, and total bicarbonate) were obtained at the beginning and end of the infusions in groups I and II. More frequent determinations were performed during the infusion and early postinfusion period in two patients of group I and in all patients of groups III–V. All assays were made in duplicate.

Statistical methods. Results obtained at the various time points of the studies were expressed as changes from the corresponding last basal value (±SEM) (Figs. 1–3). The statistical significance of these changes was evaluated within each group by a paired t test. Results of all groups were submitted to a one-way analysis of variance at each time point and whenever a difference was detected at a statistically significant level ($P < 0.05$), simultaneous pair-wise comparison between groups was made by a t test using the SE derived from the analysis of variance (16).

Preparation of material for infusion. Sodium AcAc was prepared from ethyl-AcAc (Merck-Schuchardt, München, Germany) as described (14). AcAc acid was obtained by mixing 1.6 M Na AcAc with increasing amounts of strongly acidic cation exchange resin (Amberlite IR-120, Mallinckrodt Inc., St. Louis, Mo.) under pH monitoring until the solution reached a pH of 2.6. Since the pK of AcAc acid is 3.6, 90% of the AcAc acid should be in its free form. After this ion-exchange procedure, the recovery of AcAc acid (as determined enzymatically) was only 49±2% of the expected amount, probably because of decarboxylation of part of the AcAc acid to acetone. The latter was removed by exposing the AcAc acid solution to a stream of nitrogen in an ice bath for 2 h. An indirect estimation of the amount of acetone that could contaminate the AcAc acid preparations was obtained on three occasions by the following way: Trace amounts of [3-^{14}C]ethyl-AcAc were mixed with unlabeled ethyl-AcAc at the start of the preparation procedure. The AcAc acid solutions obtained were analyzed for both their unlabeled AcAc acid content (enzymic determination) and their total radioactivity, which, besides the [^{14}C]acetoacetic acid, would include any remaining [^{14}C]acetone. The fact that the recoveries of total ^{14}C (46, 53, and 48%) were almost identical to those of AcAc acid (47, 50, and 45%) indicates that only negligible amounts of acetone could have been present in our infusion solutions. The hydrochloric acid used in studies of group IV was delivered as an isotonic solution (0.145 M) in water.

All solutions were prepared the day before the experiments, sterilized on a Millipore filter (0.22 μm; Millipore Corp., Bedford, Mass.), kept frozen at −20°C until use, and kept cool with ice during the infusion.

[2] When ALA was assayed on the perchloric filtrate of 58 plasma samples, values obtained were 28±1% higher than those obtained on the same samples without prior deproteination, but a very close correlation ($r = 0.997$) was observed between the two groups of determinations.

RESULTS

Table I shows that the five groups of subjects were comparable in age, relative body weight, and substrate concentration. Plasma levels of metabolites and immunoreactive insulin were in the accepted normal range for all groups. As stated, the relatively low basal levels of ALA can probably be accounted for by the fact that this substrate was assayed on nondeproteinized samples.

Control studies (Fig. 1). During the 11.5 h of the test, the progression of the fast in the control subjects receiving no infusion induced a mild increase in ketone levels, which remained below 1 mM, and a slow decrease in glucose levels averaging 7.7±2.1 mg/dl at the end of the experiments. Average plasma ALA concentration fell progressively, although not significantly, during the first 6.5 h of the test. There were no systematic changes in the levels of GLN and lactate.

Sodium acetoacetate infusion studies (Fig. 2). The administration of Na AcAc induced a sharp rise in both AcAc and βOHB levels, total ketone concentration tending to plateau at about 3 mM at the end of the infusion. At that time the ratio of βOHB/AcAc averaged 1.3±0.07, a value significantly lower ($P < 0.02$) than that observed during the basal period (2.3±0.07). During the first hour after completion of the infusion, there was a rapid decline in ketone levels, which did not differ significantly from those of controls during the last 6 h of the study.

The infusion of Na AcAc induced a slight alkalosis; the blood pH increased by 0.10±0.003 U above basal value ($P < 0.001$) at termination of the infusion. Corresponding increases in bicarbonate concentrations were 4.40±0.68 mM ($P < 0.001$). In the two subjects in whom blood pH was measured during both the infusion and postinfusion period, mean increases in blood pH were 0.10 at 3 h (end of the infusion), 0.10 at 4 h, 0.09 at 5 h, and 0.07 at 6 h, showing that the alkalinizing effect of Na AcAc persists for several hours after cessation of the infusion.

ALA concentration decreased during the preinfusion period and fell further after Na AcAc administration. By comparison with the last basal value, a decrease of 0.046±0.011 mM was recorded at the end of the infusion but this fall did not differ significantly from that observed in the controls (0.021±0.012 mM). Thereafter, despite the rapid regression of hyperketonemia, ALA concentration continued to fall for several hours. The mean maximal decrease below basal level reached 0.089±0.012 mM ($P < 0.001$) 2.5 h after cessation of the infusion. Significant differences ($P < 0.05$) with the control group were observed from the first to the third hour of the postinfusion period.

GLN levels remained relatively steady during the basal period and started to decrease slowly after the first hour of infusion. The mean decrease from last basal level was maximal 1 h after the end of the infusion and amounted to 0.083±0.014 mM ($P < 0.001$). Sig-

TABLE I
*Characteristics of Groups of Subjects**

Group	Infusion	n	Male/ Female	Age	BW × 100 / IBW	ALA	GLN	Glucose	Immunoreactive insulin	Total ketones	FFA
				yr		*mM*	*mM*	*mg/dl*	*μU/ml*	*mM*	*mM*
I	Na AcAc (21.5±0.9 μmol/kg/min)	13	8/5	22±1	97±3	0.24±0.02	0.75±0.03	108±2	17±2	0.11±0.02	0.65±0.06
II	Na bicarbonate (21.0±1.0 μmol/kg/min)	8	7/1	23±1	101±5	0.25±0.03	0.79±0.04	107±3	—	0.12±0.02	—
III	AcAc acid (20.2±0.8, μmol/kg/min)	8	5/3	29±4	101±3	0.19±0.02	0.72±0.04	100±3	18±1	0.17±0.03	0.76±0.06
IV	Hydrochloric acid (7.0±0.2, μmol/kg/min)	7	7/0	22±1	93±2	0.20±0.02	0.69±0.03	—	—	0.16±0.05	—
V	Controls (no infusion)	7	5/2	24±1	104±3	0.24±0.02	0.73±0.05	102±3	16±2	0.11±0.02	—

* Substrates and immunoreactive insulin concentrations represent the mean (±SEM) of four values obtained during the basal period. BW, body weight; IBW, ideal body weight.

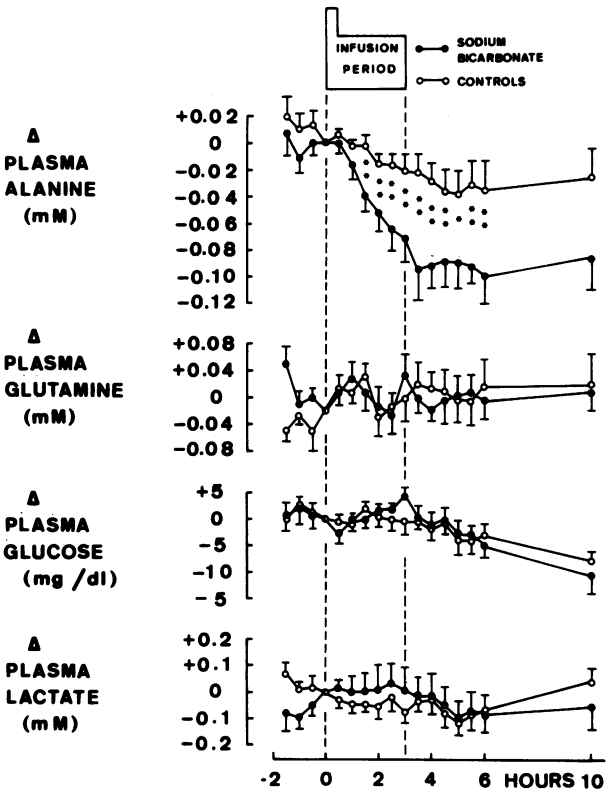

FIGURE 1 Modifications in plasma concentration (mean ±SEM) of alanine, glutamine, glucose, and lactate induced by the infusion of sodium bicarbonate in normal overnight-fasted subjects. The control subjects received no infusion. The data are expressed as changes from the last basal value. Statistical significance of differences between the two groups: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.001$.

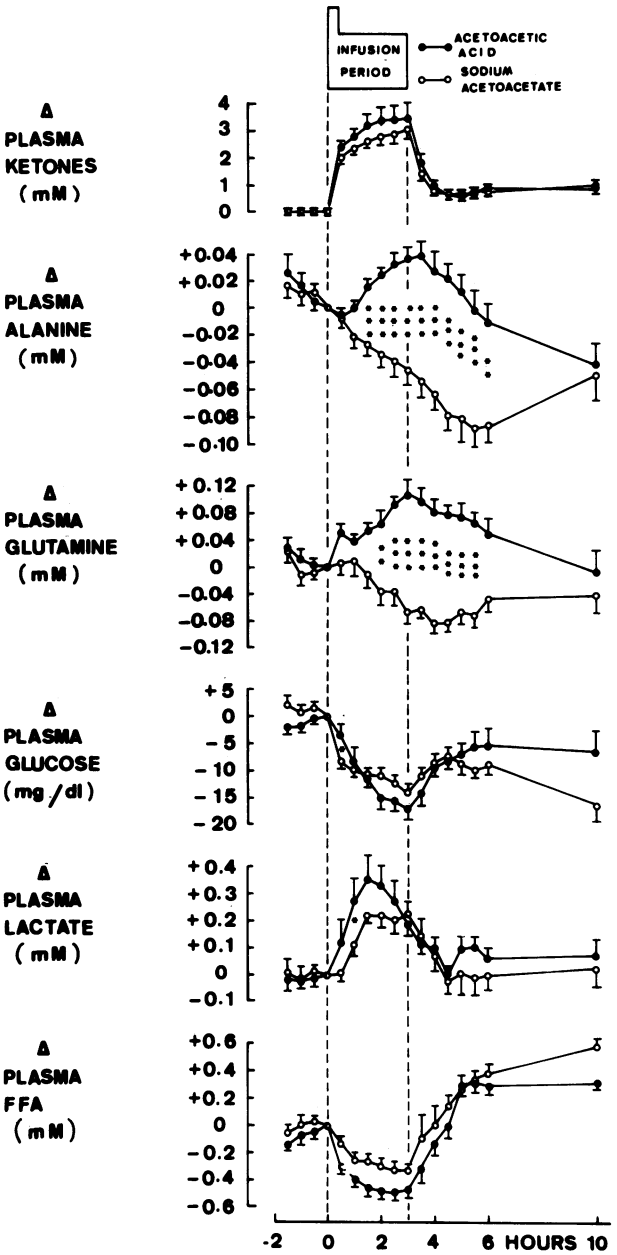

FIGURE 2 Modifications in plasma concentrations (mean ±SEM) of total ketone bodies (AcAc + βOHB), alanine, glutamine, glucose, lactate, and FFA induced by the infusion of sodium AcAc or AcAc acid in normal overnight-fasted subjects. Statistical significance of differences between the two groups as in Fig. 1.

nificant differences with the control group were observed 0.5 h ($P < 0.005$), 1 h ($P < 0.005$), 1.5 h ($P < 0.01$), and 2.5 h ($P < 0.05$) after completion of the infusion.

Plasma lactate levels rose during Na AcAc administration, a maximal increase of 0.23±0.05 mM ($P < 0.001$) above last basal value being observed at the end of the infusion. The difference with the control group was highly significant ($P < 0.005$) during the last 2.5 h of infusion.

The hypoglycemic action of Na AcAc was already apparent after 30 min of infusion, attained its maximum after 3 h (−14±2 mg/dl; $P < 0.001$) and faded out progressively during the postinfusion period. Significant differences with the control group were observed during the entire infusion period ($P < 0.005$) and the following 2 h ($P < 0.02$). Plasma immunoreactive insulin levels were not significantly influenced by Na AcAc. The ketone administration produced a decrease in FFA levels averaging 0.32±0.04 mM ($P < 0.001$) at the end of the infusion. This fall was followed by a pronounced rebound so that FFA concentrations were about 60–70% higher than basal levels during the last 4 h of the experiments (Fig. 2).

Sodium bicarbonate infusion studies (Fig. 1). Since the administration of Na AcAc induced a significant alkalinization of blood, experiments were performed in which Na AcAc was replaced by equimolar amounts

of Na bicarbonate to check whether alkalinization per se influences substrate concentration. The rise in blood pH observed at the end of the infusion (+0.070±0.008) was slightly but significantly less marked ($P < 0.05$) than that observed in the Na AcAc experiments, but the rise in blood bicarbonate (+5.13 ±0.74 mM) was not significantly different. The Na bicarbonate-induced alkalosis was associated with a fall in ALA levels that was more marked than that observed in the control study. The statistical significance of the differences between the two groups is depicted in Fig. 1. A mean maximal decrement of 0.100±0.021 mM ($P < 0.005$) was observed 3 h after termination of the infusion. The lowering effect of Na bicarbonate on ALA concentration was statistically indistinguishable from that obtained with isomolar amounts of Na AcAc, whatever time point was considered. On the other hand, Na bicarbonate was devoid of any significant effect on GLN, glucose, and lactate levels (Fig. 1).

Acetoacetic acid infusion studies (Fig. 2). The hyperketonemia induced by the infusion of AcAc acid followed a pattern very similar to that observed in the Na AcAc experiments. At no time of the study was there any statistically significant difference between total ketone body levels or βOHB/AcAc ratios in the two groups. The administration of AcAc acid was associated with a small but significant fall in blood pH that averaged 0.019±0.006 U ($P < 0.02$) at 1 h and 0.019±0.008 U ($P < 0.05$) at 2 h of infusion. At the end of the infusion and during the following 2 h, pH did not differ significantly any further from its preinfusion value. Bicarbonate concentration fell by 2.1±0.5 mM ($P < 0.005$) at 1 h, 3.3±0.7 mM ($P < 0.005$) at 2 h, and 3.1±0.8 mM ($P < 0.01$) at 3 h of infusion and returned thereafter to its basal level.

As in the control group, ALA concentration decreased during the basal period. After a delay of 30 min, the infusion of AcAc acid induced a rise in ALA levels that contrasted with the persistent fall observed in the control group. A maximal increase of 0.038 ±0.012 mM ($P < 0.02$) above last basal value was noticed 30 min after termination of AcAc acid infusion (Fig. 2). Subsequently ALA levels returned to base-line value in about 2 h and continued thereafter to fall until the end of the study. Significant differences with the control group ($P < 0.02$) were observed during the last 60 min of infusion and the initial 90 min of the postinfusion period.

A similar pattern was observed for GLN levels. Fig. 2 shows that within 30 min, the infusion of AcAc acid induced a rise in the concentration of this amino acid, which reached a maximum of +0.107±0.023 mM ($P < 0.005$) at the end of the infusion. This peak elevation was followed by a progressive return towards base-line level. These changes in GLN levels differed significantly ($P < 0.02$) from those observed in the controls during the last hour of infusion and the first hour of the postinfusion period. Thus, equimolar amounts of AcAc acid and Na AcAc, inducing the same degree of hyperketonemia have opposite effects on the plasma concentration of both ALA and GLN. Fig. 2 shows that highly significant differences exist between these two groups for both amino acids during the last 1–2 h of infusion and the subsequent 2–3 h. On the other hand, the AcAc acid infusions induced a fall in glucose and FFA concentration and an increase in lactate levels which were comparable to those observed in the Na AcAc studies (Fig. 2).

Hydrochloric acid infusion studies (Fig. 3). These experiments were designed to determine whether blood acidification per se modifies plasma ALA and GLN concentration.

After 1, 2, and 3 h of HCl infusion, blood pH decreased by 0.016±0.008, 0.024±0.007, and 0.046 ±0.011 U, respectively. The decrement at 3 h was significantly greater ($P < 0.01$) than that observed at the same time in the AcAc acid experiments in which it amounted only to 0.013±0.009 U. The fall in plasma bicarbonate induced by HCl averaged 1.5±0.4 mM at 1 h, 2.1±0.3 mM at 2 h, and 3.2±0.2 mM at 3 h of infusion, these decrements being not significantly different from those observed in the AcAc acid experiments.

Although the acidification with HCl was slightly more marked than that induced by AcAc acid, it did not reproduce the effects of the latter on ALA and GLN levels which remained unchanged as compared with

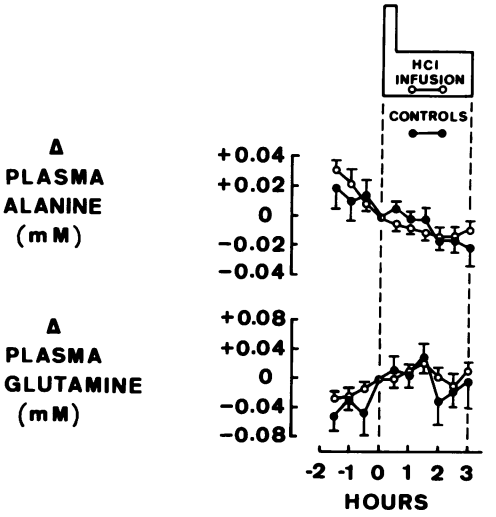

FIGURE 3 Changes in plasma concentration (mean±SEM) of alanine and glutamine in response to the infusion of hydrochloric acid. The control group received no infusion and was identical to that of Fig. 1. There were no statistically significant differences between the two groups.

the control experiments (Fig. 3). Comparison between the AcAc acid and the HCl infusion data shows that statistically significant differences between these two groups exist for ALA after 1.5 h ($P < 0.05$), 2 h ($P < 0.02$), 2.5 h ($P < 0.05$), and 3 h ($P < 0.025$) and for GLN after 2.5 h ($P < 0.005$), and 3 h ($P < 0.02$) of infusion.

DISCUSSION

Effect of ketone bodies on ALA and GLN levels. The hypoalaninemic effect of Na AcAc infusion observed in this study confirms the results obtained by Sherwin et al. (1) with Na βOHB infusions. In the two studies, the ketone bodies were administered in comparable amounts (21.5 and 23.8 μmol/kg/min, respectively) and similar decreases in ALA levels were observed (22 and 19%, respectively, after 3 h of ketone infusion). The reason why the hyperketonemia reached in our subjects (~3 mM) was about three times higher than that observed by Sherwin et al. (~1 mM) can be explained by the fact that these authors infused the racemic mixture of βOHB, the enzymatic procedure employed for determining βOHB level in plasma being specific for the D(−) isomer.

Sherwin et al. (1) discarded the possibility that the observed hypoalaninemic effect could result from the alkalinizing action of Na βOHB infusion by showing that Na bicarbonate was devoid of any significant effect on ALA concentration. Yet, the quantity of Na bicarbonate used in these control studies (one-third to one-half the amount of Na DL βOHB on a molar basis) might have been inappropriately low, since in the four subjects tested the rise in serum bicarbonate averaged 3 mM, whereas it amounted to 5 mM in the Na βOHB group. Blood pH values were not provided in these experiments. We observed in our studies that equimolar amounts of Na bicarbonate and Na AcAc are required to induce comparable changes in plasma bicarbonate and blood pH levels. Under these conditions, Na bicarbonate completely reproduced the hypoalaninemic effect of Na AcAc. This observation suggests that the changes in ALA levels induced by Na AcAc are related to its alkalinizing properties rather than to hyperketonemia itself. The existence of a delay of several hours between the end of the infusion of Na AcAc and its maximal hypoalaninemic effect is consistent with this interpretation. Indeed, repeated determinations of blood pH and bicarbonate levels during the postinfusion period of two Na AcAc experiments have shown that the alkalinizing effect of Na AcAc lasts several hours longer than the hyperketonemia.

To eliminate the interference of the unphysiological blood alkalinization induced by the infusion of the sodium salt of keto acids, we performed a series of experiments using free AcAc acid. The major contribution of this study is to demonstrate that under these conditions ketone bodies induce an increase instead of a decrease in plasma ALA and GLN levels and in our view, this effect represents the genuine action of ketone bodies on these amino acids. The possibility that the hyperalaninemic and hyperglutaminemic effects of AcAc acid represent a nonspecific response to blood acidification is unlikely since it could not be reproduced with HCl infusions (Fig. 3).

The mechanism of the AcAc acid induced rise in ALA concentration has not been explored in the present work. Theoretically it might correspond to increased production or decreased splanchnic extraction or both. A direct inhibitory effect of ketones on hepatic ALA uptake can probably be excluded because the liver lacks the enzymes for using ketones (17). An insulin-mediated inhibition of hepatic ALA uptake could be taken into consideration, since it is possible (18) although not certain (19–21) that insulin restrains splanchnic ALA extraction in man. In this respect, it should be noted that under our experimental conditions, no insulinotropic effect of AcAc acid could be detected in peripheral blood. This does not rule out that a modest increase in insulin concentration might have occurred in portal vein blood (22).

The comparative effects of Na AcAc, Na bicarbonate, and AcAc acid infusions on GLN levels are somewhat puzzling. Indeed, the decrease in GLN levels observed in the Na AcAc experiments could not be reproduced with equimolar amounts of Na bicarbonate, suggesting that Na AcAc has hypoglutaminemic effects that are independent of changes in pH. This observation is difficult to reconcile with the hyperglutaminemic action of free AcAc acid.

As in the case of ALA, the possibility that the AcAc acid-induced rise in GLN concentration might result from a decreased removal should be considered. Indeed, it has been shown in dogs that ketone bodies inhibit the uptake of GLN by the kidney (23), an important site of removal of this amino acid (24). However, since this effect has been observed with the sodium salt as well as with the acid form of ketone bodies (23), it cannot account for our observation that Na AcAc and AcAc acid have opposite effects on GLN concentration.

A possible explanation for the increase in ALA and GLN levels induced by AcAc acid infusion is a stimulation of the release of these amino acids by muscle that is a major site of their overall production (24). However, such an effect could not be documented in in vitro studies using the isolated diaphragm (25) or the perfused hindquarter of the rat (26). Obviously, the possibility of an increased production by other

tissues should also be considered. It is tempting to speculate that some common biochemical mechanism underlies the concomitant increase in ALA and lactate occurring in the AcAc acid infusion experiments, but the existence of such a mechanism seems unlikely because a similar rise in lactate was observed during infusions of Na AcAc, which lowered ALA levels. Preliminary data from our laboratory indicate that the increase in amino acids associated with AcAc acid infusions is not specific for ALA and GLN. A rise was also observed for many other amino acids, suggesting that AcAc acid might stimulate muscular proteolysis.

Relationship between changes in amino acids and glucose levels during the infusion of ketone bodies. It is well known that infusions of Na AcAc (27) or Na βOHB (28, 29) in man and other species induce a decrease in plasma concentration of glucose, FFA, and glycerol. Our results are consistent with these data and show that the same effect is observed with infusions of AcAc acid. The hypoglycemic effect of ketones is related to a decrease in hepatic glucose output (29), which seems independent of a stimulation of insulin secretion since this hypoglycemic action can be demonstrated in insulin dependent diabetics (30, 31).

Sherwin et al. (1, 30) suggested that the hypoglycemic effect of ketones might be related to the decrease in the concentration of ALA, a major hepatic glucose precursor. In the light of our experiments, this hypothesis seems unlikely because AcAc acid infusion increases ALA levels and yet induces an hypoglycemic effect identical to that of Na AcAc. This consideration is of interest with respect to the mechanism of the hypoalaninemia reported in ketotic hypoglycemia (32), a common hypoglycemic syndrome of children. According to our data, the hypothesis that hypoalaninemia might be a consequence of the hyperketonemia (1), should probably be rejected.

Possible role of ketone bodies in the regulation of amino acid metabolism during fasting and other ketotic states. It is generally accepted that, by comparison with the postabsorptive state, brief starvation (a few days) is characterized by a stimulation of the muscular production (5) and the splanchnic uptake of ALA (33), which represents quantitatively the most important glucogenic amino acid. Concomitantly, gluconeogenesis is stimulated (34) and nitrogen is lost in urine at the rate of about 12 g/24 h (35, 36). Ketone production is as great as that observed after a prolonged fast (34, 37) and ketonemia is already significantly elevated (35, 37). A different picture is observed after several weeks of fast. At that time, the muscular release and the plasma concentration of ALA and GLN are markedly decreased (2, 38), gluconeogenesis is depressed (36) and the urinary nitrogen excretion is reduced to about one-third of that observed during early starvation (36). Ketone bodies are markedly elevated (36, 37) and their oxidation accounts for an important proportion of the energy needs of the brain (39).

As yet, there is no satisfactory explanation for the biphasic changes in glycogenic amino acid production occurring during progression of the fast. The increased output of ALA observed in brief starvation is usually ascribed to the fall in insulin and to the rise in glucagon levels (24). However, this explanation is in contradiction with the observation of Pozefsky et al. (40) who showed that physiologic increases of either hormone is devoid of any significant effect on ALA balance across human forearm. In vitro studies have also failed to demonstrate a significant effect of insulin (41, 42) and glucagon (41) on muscular ALA or GLN release. With regard to prolonged fasting, Sherwin et al. (1) raised the hypothesis that the reduction in ALA and GLN output, and the nitrogen sparing observed under these conditions are related to the hyperketonemia. This conception of the regulatory role of ketone bodies in amino acid metabolism during prolonged fasting can be questioned for the following reasons: Firstly, our data indicate that the hypoalaninemic effect of ketones described by Sherwin et al. represents an experimental artifact related to the alkalinizing effect of the Na βOHB infusion used. Secondly, even if ketone bodies had the property of reducing the muscular output of ALA, it seems unlikely that several weeks of fast are necessary for this effect to become operative, since at that time, the muscular uptake of ketones is markedly reduced (6), if not completely abolished (7). It is recognized that Sherwin et al. were able to reduce the urinary nitrogen output of starved subjects with prolonged infusions of Na βOHB. However, before these data can be interpreted as an argument for a protein sparing effect of ketone bodies, it would be necessary to show, firstly, that control infusions with equimolar amounts of Na bicarbonate do not produce the same effect, and secondly, that the inhibitory effect of ketone bodies persists if ketones are delivered in their physiological free acid form. In our studies, the relatively short duration (3 h) of the AcAc acid infusion precluded a reasonably accurate estimation of possible changes in urinary nitrogen. In fact, several studies provide indirect evidence that endogenous ketones do not promote nitrogen sparing. For instance, it has been shown in surgical patients receiving intravenous parenteral nutrition containing amino acids that the hyperketonemia resulting from the isocaloric replacement of glucose by lipids did not improve nitrogen balance (43, 44). As stated, similar conclusions were reached by Yang et al. (8) in obese subjects submitted to a hypocaloric regimen. Indeed, the replacement, under isoproteic iso-

caloric conditions, of a nonketogenic diet by a ketogenic diet increased, although not significantly, the nitrogen deficit.

Obviously, the possible role of ketone bodies in the regulation of glucogenic amino acid transport during fasting has to be reevaluated in the light of the present results. Assuming that the rise in ALA and GLN levels observed during infusions of AcAc acid reflects an increased muscular production of these amino acids, it seems reasonable to suggest that hyperketonemia participates to the enhanced ALA release associated with short-term fasting. One could also anticipate that this ketone-induced overproduction of ALA (and GLN) fades out with time, in so far as muscular ketone uptake decreases with progression of the fast. Such reasoning would provide an explanation for the decreased muscular output of glucogenic amino acids and the reduced protein breakdown characteristic of prolonged starvation. However, experiments with infusions of AcAc acid should be performed in prolonged-fasted subjects to ascertain this possibility. According to this view, the role of ketone bodies in regulating glucogenic amino acid transport would be predominant in short-term starvation. At that stage, when both ketogenesis and gluconeogenesis are markedly stimulated, ketone bodies might indirectly contribute in supplying the liver and the kidney with gluconeogenic substrates.

The infusion of exogenous ketones represents a useful tool that has been widely used in searching for specific roles of ketone bodies in metabolic regulations. However, one should remain cautious with regard to the physiological significance of data obtained from such infusion studies. Administration of exogenous βOHB or AcAc lowers FFA levels markedly (Fig. 2) (28), inhibits FFA oxidation by various tissues (45), and stimulates to some degree insulin secretion (22). On the contrary, all physiological (e.g., fasting, fat diet) or pathological (e.g., diabetes, ketotic hypoglycemia) states of hyperketonemia are characterized by high FFA levels, high rates of FFA oxidation, and low insulin concentrations. It is thus conceivable that in spontaneous ketosis, ketone bodies might have metabolic actions that could be quantitatively or even qualitatively different from those observed in artificially induced hyperketonemia.

ACKNOWLEDGMENTS

The authors express their gratitude to M. A. Neef-Debel for expert technical assistance. Thanks are also due to D. Calay, nurse of the Metabolic Unit for assistance with blood sampling, to Dr. R. Naeije and Dr. E. Van Thiel for placing the catheters, to Dr. M. Staquet and Mrs. O. Dalesio for assistance in statistical analysis, and to C. Demesmaeker for secretarial help.

This research was supported by the Fonds de la Recherche Scientifique Médicale (grant 3/4537/76).

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