FEBS 18815 FEBS Letters 412 (1997) 179–182

The pH-dependent reciprocal changes in contributions of ADP/ATP antiporter and aspartate/glutamate antiporter to the fatty acid-induced uncoupling

Victor N. Samartsev[a,b], Elena N. Mokhova[b], Vladimir P. Skulachev[b,]*

[a]*Mari State University, Yoshkar-Ola, Russia*
[b]*A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russia*

Received 16 May 1997

Abstract **The pH effect on carboxyatractylate-, glutamate- and aspartate-induced recoupling of palmitate-uncoupled rat liver mitochondria has been studied. Stimulation of respiration by low palmitate concentrations (5–20 μM) in the presence of 3 mM $MgCl_2$ is shown to be pH-independent within the 7.0–7.8 range. The recoupling effect of glutamate (or aspartate) decreases and that of carboxyatractylate increases with increase in pH. The recoupling effect of a combination of carboxyatractylate and glutamate (aspartate) appears to be constant at these pH values, being as high as about 80%. It is concluded that uncoupling by low palmitate in liver mitochondria is mediated mainly by ATP/ADP and aspartate/glutamate antiporter.**

Key words: Fatty acid; Uncoupler; Recoupler; ATP/ADP antiporter; Aspartate/glutamate antiporter; Carboxyatractylate; Glutamate; Aspartate; Liver mitochondria

1. Introduction

The ATP/ADP and aspartate/glutamate antiporters are assumed to be involved in fatty acid-induced uncoupling in liver mitochondria [1–5]. This assumption is based on the fact that substrates of these anions carriers (ADP, glutamate and aspartate) and their inhibitors (carboxyatractylate and diethylpyrocarbonate) cause recoupling when added to palmitate-uncoupled mitochondria.

At pH 7.4, the recoupling effects of carboxyatractylate and glutamate (or aspartate) were almost equal [5]. It is not known what happens at other pH values. In thermogenine proteoliposomes, the suppression of H^+ transport activity by GTP, ADP, ATP and their derivatives, decreases when pH increases from 6.5 to 7.5 [6].

As for ADP/ATP antiporter, in the range between pH 6 and 8, changes in pH do not affect the rate of ADP/ATP transport but influence the interaction of the ATP/ADP antiporter with its inhibitors [7,8]. For the aspartate/glutamate antiporter, variable data have been reported, depending on experimental conditions. The pronounced pH-dependence was shown for glutamate/glutamate exchange in the aspartate/glutamate antiporter proteoliposomes. At pH 7.4 K_m for glutamate was about 10 times higher than at pH 6.5 [9].

In this study, we have found that in incubation medium with $MgCl_2$, the recoupling effect of glutamate (aspartate) decreases and this of carboxyatractylate increases when pH changes from 7.0 to 7.8. Within this pH range, the combined recoupling effect of carboxyatractylate and glutamate (aspartate) is shown to remain constant.

2. Material and methods

Mitochondria were isolated from the liver of a white rat (180–220 g). The isolation medium contained 250 mM sucrose, 1 mM EGTA, 5 mM MOPS–KOH (pH 7.4). The homogenate was centrifuged at 700×*g* for 10 min. Mitochondria were sedimented at 10 000×*g* for 10 min, resuspended in 1 ml of isolation medium supplemented with BSA (3 mg/ml), diluted with 30 ml of isolation medium without BSA, and centrifuged at 10 000×*g* for 10 min. The mitochondrial pellet was resuspended in the isolation medium. The final mitochondrial suspension contained about 60 mg protein/ml.

Mitochondrial protein was measured using the biuret method.

Oxygen consumption was recorded by a Clark-type oxygen electrode and LP-9 polarograph. The incubation medium contained 250 mM sucrose, 5 mM potassium succinate, 2 μM rotenone, 3 mM $MgCl_2$, 0.5 mM EGTA, and 5 mM MOPS–KOH; pH was varied from 7.0 to 7.8. In all the experiments, oligomycine (2 μg/ml) was

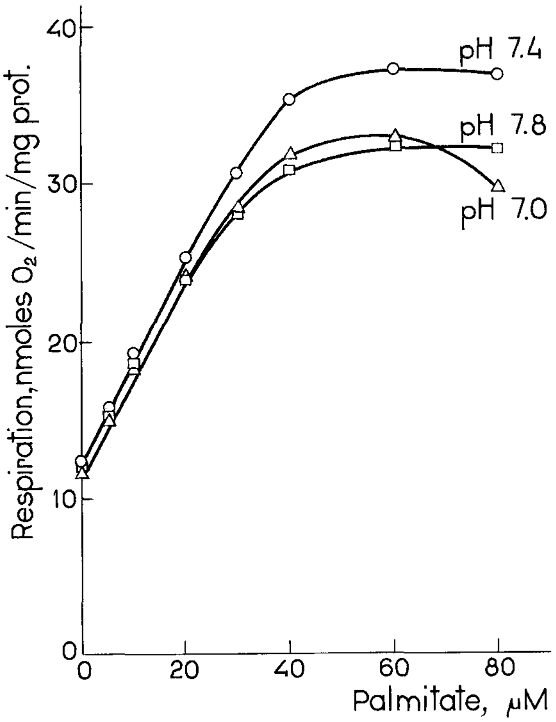

Fig. 1. Concentration dependence of palmitate-induced uncoupling at different pH values. For experimental conditions, see Section 2.

---

*Corresponding author. Fax: (7) 095-939-03-38.
E-mail: skulach@head.genebee.msu.su

Abbreviations: DNP, 2,4 *p*-dinitrophenol; EGTA, ethylene glycol-*bis*-(2-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; BSA, bovine serum albumin

PII S0014-5793(97)00667-4

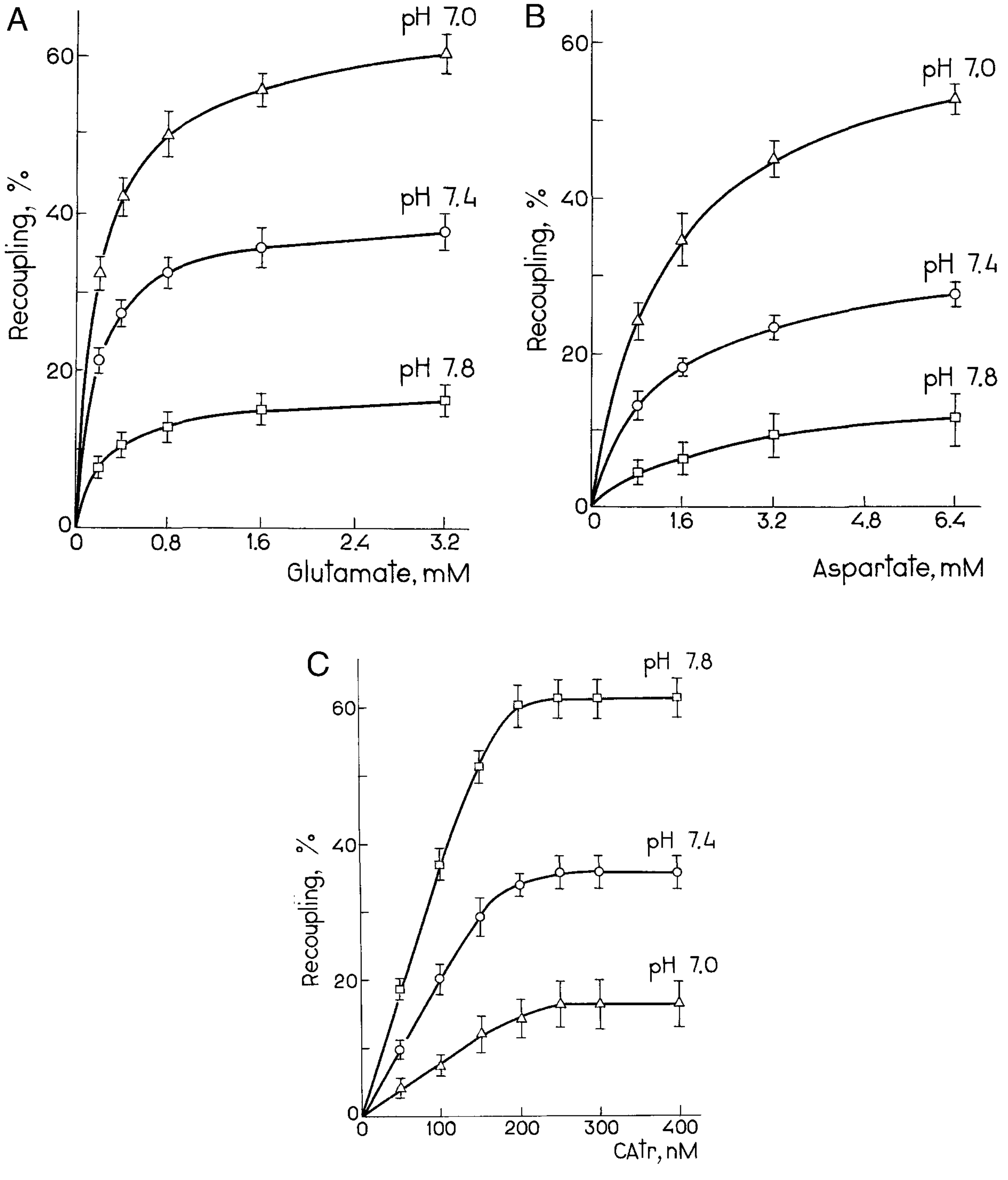

Fig. 2. Concentration dependence of glutamate (A), aspartate (B) and carboxyatractylate (C) recoupling effects at different pH. All the recouplers were added to mitochondria after 15 μM palmitate. For other conditions, see Section 2. The recoupling was measured as a decrease in the rate of respiration uncoupled by palmitate.

added just after mitochondria. The concentration of mitochondrial protein was about 1 mg/ml.

The fatty acid-induced uncoupling was estimated by a stimulation of oxygen consumption after addition of palmitate in the presence of oligomycine. The recoupling effects of carboxyatractylate, glutamate and aspartate were measured by inhibition of the palmitate-stimulated respiration. Previous experiments showed that this inhibition was accompanied by increase in membrane potential and was abolished by DNP [4,5].

MOPS, palmitic acid, oligomycine, succinate, glutamate, carboxyatractylate, EGTA, and delipidized BSA were from Sigma; rotenone, from Serva; DNP, from Fluka; and aspartate from Reanal. Aspartate was twice recrystallized from bidistilled water. Sucrose was twice precipitated from a concentrated solution in bidistilled water with distilled ethanol. Palmitic acid was dissolved in ethanol up to 10 mM concentration.

3. Results and discussion

As was previously shown in this group, the recoupling effect of glutamate is dependent upon ion composition of the incubation medium, namely addition of $MgCl_2$ to sucrose medium

Table 1
Effects of carboxyatractylate and glutamate on stimulation of respiration by palmitate at different pH values (means+standard error, *n*=6–7)

Respiration rates (nmol O_2/min/mg prot)			
Additions	pH 7.2	pH 7.4	pH 7.8
–	9.9 ± 0.4	10.8 ± 0.3	11.6 ± 0.4
Palm	22.8 ± 1.4	24.7 ± 0.8	24.3 ± 0.7
Palm+CAtr	20.8 ± 1.4	19.5 ± 0.4	16.5 ± 0.3
Palm+CAtr+Glu	12.7 ± 0.7	14.1 ± 0.3	14.3 ± 0.3
Palm+CAtr+Glu+DNP	52.4 ± 1.7	56.2 ± 1.7	45.0 ± 1.8

For experimental conditions, see Section 2.
Additions: Palm, 15 μM palmitate, CAtr, 1 μM carboxyatractylate, Glu, 2 mM glutamate, DNP, 50 μM DNP.

increased recoupling [10]. In the presence of 3 mM $MgCl_2$, stimulation of respiration by low palmitate concentrations (5–20 μM) proved to be pH-independent within the 7.0–7.8 range (Fig. 1). In all of the following experiments, 15 μM palmitate was used.

At pH 7.0, suppression of palmitate-induced uncoupling by carboxyatractylate was small. The recoupling effect of carboxyatractylate increased with an increase in pH. At pH 7.8, carboxyatractylate suppressed about 60% of the palmitate-induced increase of the respiration rate in the presence of oligomycin (Table 1).

On the other hand, the effect of glutamate recoupling was small at pH 7.8. It increased with a decrease in pH, and at pH 7.0 glutamate suppressed 60% of the palmitate-induced increase in the respiration rate (Table 1). The same results were obtained when aspartate was added instead of glutamate (not shown).

The recoupling effect of glutamate increased with an increase in the glutamate concentration and reached maximum at about 1.6 mM glutamate (Fig. 2A). Aspartate produced recoupling at higher concentrations than those of glutamate (maximum at about 6 mM) (Fig. 2B). The concentrations of carboxyatractylate, glutamate and aspartate, at which the half-maximal recoupling effects were observed, proved to be pH-independent (Fig. 2).

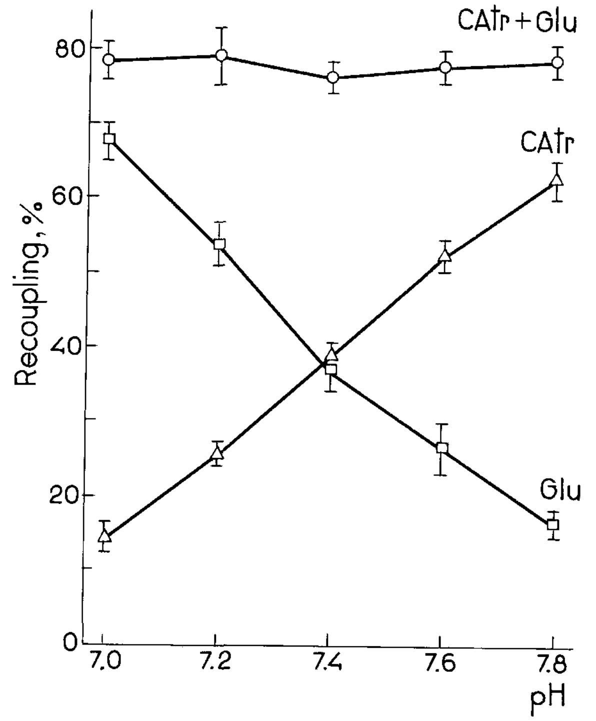

Fig. 3. Recoupling effect of glutamate, carboxyatractylate and their combination at different pH values. Additions: CAtr, 1 μM carboxyatractylate; Glu, 2 mM glutamate. For other conditions, see Section 2.

The recoupling effect increased after addition of glutamate in the presence of carboxyatractylate (Table 1) or after addition of carboxyatractylate in the presence of glutamate (not shown). The same result was obtained when aspartate was used instead of glutamate (not shown). It should be stressed that the maximal recoupling effect of carboxyatractylate with glutamate (or with aspartate) was constant within all the pH ranges studied (Fig. 3).

A pronounced recoupling effect of carboxyatractylate at pH 7.8 is noteworthy. In fact, rather low carboxyatractylate recoupling measured at pH 7.4 in liver mitochondria [11] is explained not only by a low amount of the ATP/ADP antiporter (as compared with heart mitochondria), but also by non-optimal pH value.

The ATP/ADP antiporter was proposed to transfer the fatty acid anions using the same machinery as the nucleotide anions [3]. Lysine residues were shown to be involved in nucleotide transport by the ATP/ADP antiporter [12]. If lysines are involved in the uncoupling effect of fatty acids, one may suggest that the deprotonation of these groups at high pH is responsible for the observed pH-dependence of palmitate-induced uncoupling mediated by the ATP/ADP antiporter.

On the other hand, a histidine residue was postulated to participate in proton binding by the aspartate/glutamate antiporter [9]. If protonated histidine somehow takes part in the uncoupling effect of fatty acids, this may explain why uncoupling mediated by the aspartate/glutamate antiporter is more effective at pH 7.0 than at pH 7.8.

The results obtained also support the proposal that the fatty acid-induced uncoupling in liver mitochondria is mediated by anion carriers. They are in line with a recent publication that the dicarboxylate carrier also contributes to fatty acid-induced uncoupling but this contribution is rather small [13].

Acknowledgements: This study was supported by Project N 96-04-49822 of the Russian Foundation of Basic Research.

References

[1] Andreyev, A.Yu., Bondareva, T.O., Dedukhova, V.I., Mokhova, E.N., Skulachev, V.P., Tzofina, L.M., Volkov, N.I. and Vygodina, T.V. (1989) Eur. J. Biochem. 182, 585–592.
[2] V.P. Skulachev, Membrane Bioenergetics, Springer, Berlin, 1988.
[3] Skulachev, V.P. (1991) FEBS Lett. 294, 158–162.

[4] Bodrova, M.E., Markova, O.V., Mokhova, E.N. and Samartsev, V.N. (1995) Biochemistry (Moscow) 60, 1027–1033.

[5] Samartsev, V.N., Smirnov, A.V., Zeldi, I.P., Markova, O.V., Mokhova, E.N. and Skulachev, V.P. (1979) Biochim. Biophys. Acta 1319, 251–257.

[6] S.-G.H. Huang, M. Klingenberg, 35 (1996) 7846–7854.

[7] Vignais, P.V. (1976) Biochim. Biophys. Acta 456, 1–38.

[8] P.V. Vignais, M.R. Block, F. Boulay, G. Brandolin, G.J.-M. Lauquin, in V. Bengha (Ed.), CRC Press, Paris, Structure and Properties of Cell Membranes, Vol. 11, 1985, pp. 139–179.

[9] Dierks, T., Riemer, E. and Kramer, R. (1988) Biochim. Biophys. Acta 943, 231–244.

[10] V.N. Samartsev, E.N. Mokhova, Biochem. Mol. Biol. Int., 1997, in press.

[11] Shonfeld, P. (1990) FEBS Lett. 264, 246–248.

[12] Bogner, W., Aquila, H. and Klingenberg, M. (1986) Eur. J. Biochem. 161, 611–620.

[13] Wieckowski, M.R. and Wojtczak, L. (1997) Biochem. Biophys. Res. Commun. 232, 414–417.