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The influence of age on the calcium-efflux pathway and matrix calcium buffering power in brain mitochondria

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The variations with age of the ruthenium red-insensitive calcium efflux rate have been studied in rat brain mitochondria. Both H^+- and Na^+-dependent effluxes are decreased with age when expressed as a function of calcium taken up in mitochondria incubated in the presence of 0.8 mM inorganic phosphate (P_i) and 0.2 mM ADP. However, the age-dependent differences in calcium efflux rates disappear when mitochondria are incubated in the absence of ADP and P_i. It is suggested that the decrease in efflux rate observed with age corresponds to an increased calcium buffering power of the mitochondrial matrix due to an increase in mitochondrial P_i. The causes of the increased P_i accumulation in old-rat-brain mitochondria are yet unknown but possibly not due to differences in the P_i efflux. The results suggest that the age-dependent lowering of the free calcium concentration in the brain mitochondrial matrix together with the reduced activity of the calcium uniporter (Vitórica, J. and Satrústegui, J. (1986) Brain Research 378, 36–48) could lead to an impaired activation of mitochondrial dehydrogenases after a rise in cytosolic calcium.

Introduction

The synthesis of acetylcholine in rodent and human brain is reduced during ageing and in patients with dementia [1–4]. The calcium-dependent release of this neurotransmitter is similarly affected [5]. The deficiencies in calcium-dependent release of acetylcholine are associated with a reduction of calcium uptake in synaptosomes [6]. Recent evidence indicates that calcium uptake in mitochondria is markedly reduced during ageing and this defect is involved in the decrease in calcium accumulation in synaptosomes [7,8]. The impairment of calcium uptake in mitochondria could lead to an altered distribution of calcium within the nerve cell under depolarizing conditions, and, as a consequence, to a block of calcium influx.

The present work is focused on a complementary aspect of the interaction of calcium with mitochondria, namely, on the pathway for calcium efflux. The calcium efflux pathway provides a mean to regulate the mitochondrial free calcium concentration in a way independent of the uptake process. This is important, since the levels of mitochondrial free calcium are critical factors for the regulation of a number of mitochondrial enzymes involved in energy metabolism [9,10].

Brain glucose metabolism decreases in aged humans with pronounced cognitive impairments [11,12] and in old rodents [13,6]. However, in vitro studies have shown that the activity of a number of enzymes involved in energy metabolism is only

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Abbreviation: P_i, inorganic phosphate.

slightly affected by ageing, except for a decrease in NAD-isocitrate dehydrogenase (Refs. 14–16 and references therein). Hence, it is possible that the impairment in energy metabolism is secondary to changes in free mitochondrial calcium levels and/or calcium efflux. To explore this possibility we have carried out a study of the effects of age on the activity of the calcium efflux pathway in rat brain mitochondria. The calcium buffering power of the mitochondrial matrix has also been investigated.

Our results show that the rate of calcium egress via H^+- or Na^+-dependent ruthenium red insensitive calcium efflux pathways is reduced during ageing and that this reduction is brought about by changes in mitochondrial free calcium concentration. After studying the variations in phosphate content and efflux with age it is concluded that differences in the phosphate concentration of mitochondria are responsible for the decrease in mitochondrial free calcium concentration.

Materials and Methods

Male Wistar rats fed 'ad libitum' on a standard laboratory diet were used throughout this study. A fraction of mixed synaptosomal and 'free' brain mitochondria was prepared according to the method of Nicholls [17] with the modifications described before [18,19].

Measurement of the efflux of Ca^{2+} from brain mitochondria

Ca^{2+} efflux to the medium was measured using the metallochromic indicator Arsenazo III [20] and an Aminco DW2a dual-wavelength spectrophotometer, at the wavelength pair 675–685 nm. Mitochondria (2 mg protein) were added to 2.5 ml 'incubation medium' (0.32 M mannitol, 10 mM Tris/HCl, 20 mM KCl, 0.1% bovine serum albumin (fatty acid free), pH 7.4) containing 0.2 mM Arsenazo III (purified according to Ref. 20) 2.5 mM succinate (potassium salt) 2.4 μM rotenone, and different additions as indicated in the figures. A calibrated addition of $CaCl_2$ was made to provide the desired loading. After Ca^{2+} additions, the uptake was nearly complete in about 2–3 min. The active influx was stopped by adding ruthenium red (1.2 μM) and the initial rate of absorbance change was recorded. The amount of intramitochondrial Ca^{2+} (Ca^{2+} available for release) was estimated from the total absorbance change produced by the addition of 10 mM NaCl and 0.2 μM A23187. The amount of mitochondrial Ca^{2+} taken up was calculated from the absorbance change, before and after $CaCl_2$ addition.

Measurement of mitochondrial P_i content and P_i fluxes

Mitochondria were incubated at 25°C in 'incubation medium' with 2 mg mitochondrial protein/ml, 2.5 mM succinate (K^+ salt) and 2.4 μM rotenone (for further additions, see figure legends). At the times indicated in the figures 0.5 ml samples of the incubation were transferred into centrifuge tubes containing ice-cold *N*-ethylmaleimide (100 nmol/mg protein) and centrifuged for 1.5 min in an Eppendorf S414 table centrifuge. The pellet was rinsed with ice-cold 'incubation medium' and extracted with 10% trichloroacetic acid. P_i content was determined by method of Lanzetta et al. [21]. Mitochondrial disruption during the experiments of P_i efflux determined as described previously [18], was non detectable.

Other methods

The mitochondrial membrane potential was determined through the distribution of tetraphenylphosphonium (TPP^+) with the use of a TPP^+-selective electrode [18]. Proteins were determined by the biuret's method. The comparison of the slopes of the concentration dependent phases of calcium release (Figs. 1 and 2) was done by the F-test for difference between two regression coefficients [22]. The comparison between the means of different groups of data and between plateau values of Ca^{2+} efflux rates (Figs. 2 and 4 and Table I) were evaluated by the Student's *t* test.

Results

Ca^{2+}-efflux in brain mitochondria from adult and old rats

It is known that in liver and brain mitochondria, the rate of ruthenium red insensitive Ca^{2+} efflux is increased by incubation with P_i in a medium free of adenine nucleotides and is associated with a

release of P_i from mitochondria. However, the addition of ADP to the incubation medium results in an inhibition of phosphate release and a corresponding inhibition of Ca^{2+} efflux [18,23–25]. Since under these conditions the mitochondrial membrane potential is perfectly stable (not shown) the release of Ca^{2+} observed after ruthenium red addition truly estimates the independent efflux pathway.

Fig. 1 shows that the rate of ruthenium red insensitive Ca^{2+} efflux from brain mitochondria incubated in the absence of phosphate and adenine nucleotides and with limited calcium loads, is concentration dependent within the range of concentrations used, and essentially unaltered with ageing. As observed (Fig. 1B), ADP addition to the incubation medium results in a decrease of the calcium efflux rate that is more pronounced in old than in adult brain mitochondria.

Fig. 2 shows the rate of ruthenium red insensitive Ca^{2+} efflux from brain mitochondria incubated with P_i and ADP. Under these conditions mitochondria can take up, and retain high calcium loads [26], and therefore, the range of Ca^{2+} loads at which efflux can be studied is much larger.

When incubated with 0.8 mM P_i and 0.2 mM ADP, the rate of Ca^{2+} efflux from Ca^{2+}-loaded mitochondria is concentration dependent below 20–25 nmol Ca^{2+}/mg protein, and becomes constant at Ca^{2+} loads higher than these values (Fig. 2A). The behaviour of old-rat-brain mitochondria incubated under these conditions is clearly different from that of adult animals. Whereas the concentration dependent and independent phases can still be clearly observed, the absolute rates of Ca^{2+} efflux are smaller over the whole range of Ca^{2+} concentrations used.

Fig. 2B shows that the Na^+-activated Ca^{2+} efflux in brain mitochondria incubated with ADP and 0.8 mM P_i shows a similar dependency towards the Ca^{2+} load and is equally affected by ageing.

The calcium-buffering power of the mitochondrial matrix

The decreased rate of Ca^{2+}-efflux in old-rat-brain mitochondria could reflect a decrease in free matrix Ca^{2+} concentration or changes in the carrier activity.

An indirect way to learn about variations in the free mitochondrial calcium concentration is to compare calcium efflux rates at various calcium loads. By using a calcium ionophore (A23187) to promote the efflux of calcium from mitochondria,

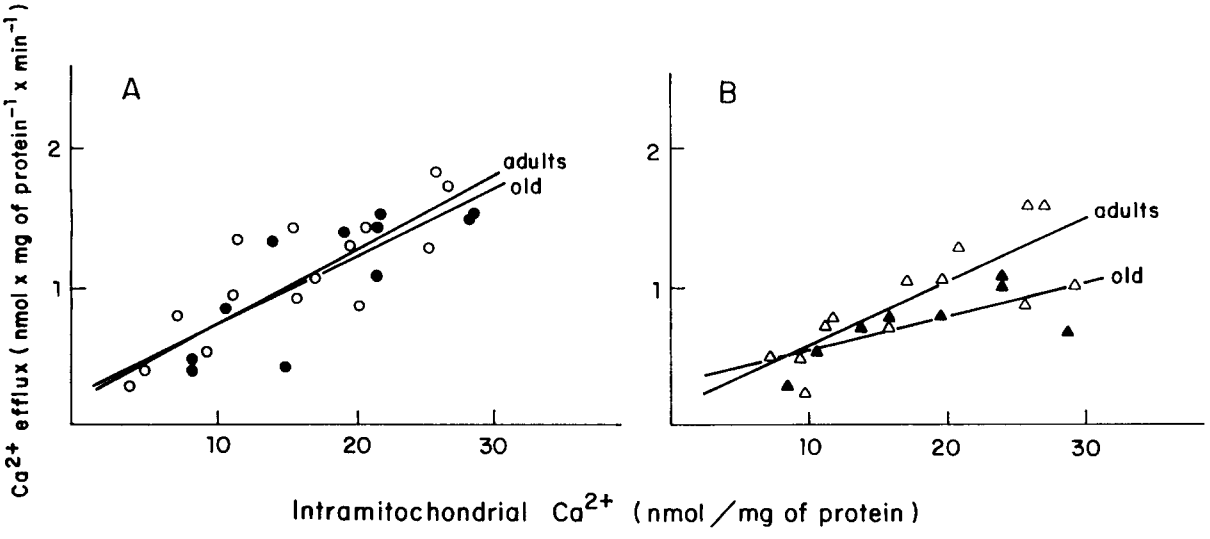

Fig. 1. Effect of ADP and age on the ruthenium red insensitive Ca^{2+} efflux rate in rat-brain mitochondria. Adult (○, △) and 24-months-old rat brain mitochondria (●, ▲) (2 mg protein) were incubated with succinate (K salt), rotenone and increasing amounts of Ca^{2+} in the presence (B) or absence (A) of 50 μM ADP. The results are individual data obtained in seven different experiments. The lines were adjusted by the least-squares method.

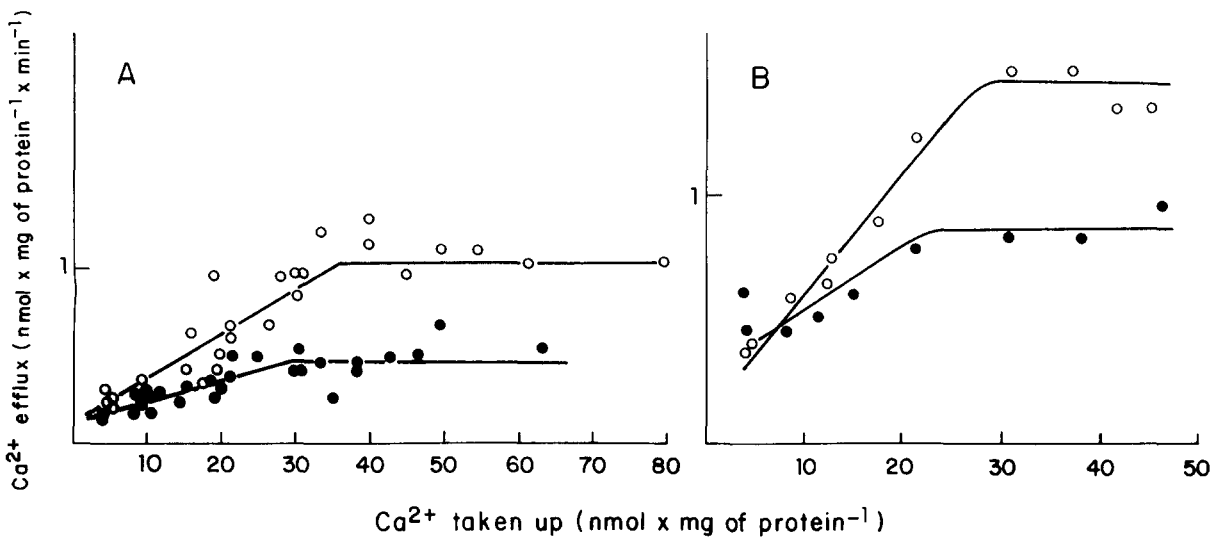

Fig. 2. H^+- and Na^+-dependent Ca^{2+} efflux rates in brain mitochondria incubated with 0.2 mM ADP and 0.8 mM P_i. Adult (open symbols) and 24-months-old rat brain mitochondria (closed symbols) were incubated with succinate (K salt), 0.4 nmol oligomycin/mg protein, rotenone and increasing amounts of Ca^{2+}, in the presence (B) or absence (A) of 10 mM NaCl. The Ca^{2+}-efflux rates are plotted against the calcium load taken up by mitochondria prior to ruthenium red addition. The slopes of the rate of Ca^{2+} efflux vs. Ca^{2+} load showed significant differences, when compared by the "F" test (see Materials and Methods) between adult or old rats ($f < 0.01$). The differences between the plateau values of old and adults were significant ($p < 0.0005$).

the calcium efflux rate observed becomes free from interferences due to carrier-mediated effects, and depends exclusively on the concentration of free calcium in mitochondria [27,18].

Following this approach we have shown previously that the variations of free calcium concentration in mitochondria incubated in the presence of P_i and ADP are, as those of the ruthenium-red insensitive calcium efflux, biphasic. There is a calcium-dependent increase at low calcium loads below 25–30 nmol mitochondrial Ca^{2+}/mg protein, and a plateau phase at higher values. In old animals, the plateau phase was lower and the slope of the calcium-dependent phase was also smaller, indicating that the buffering power of the mitochondrial matrix is higher in old animals [8]. However, endogenous calcium could be different in both mitochondrial preparations, and this could lead to an underestimation of the mitochondrial calcium content, specially at low calcium loads. Therefore we have investigated the dependence of the calcium efflux rate on the calcium available for release (see Methods).

Fig. 3 shows that the concentration-dependent rate of calcium efflux promoted by the addition of

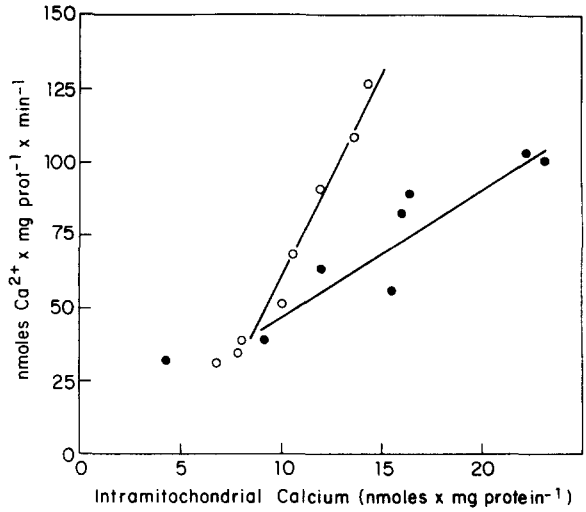

Fig. 3. Differences between the Ca^{2+} buffering power of the mitochondrial matrix in old and adult rats. Adult (○) and 24-months-old rat brain mitochondria (●) were incubated with succinate (K salt), 0.4 nmol oligomycin/mg protein, rotenone and increasing amounts of Ca^{2+}. The buffering power was determined from the rate of Ca^{2+} efflux initiated by the simultaneous addition of rutenium red, 10 mM NaCl and 0.2 μM A23187 and plotted against the amount of intramitochondrial calcium (see Materials and Methods). The data are the results of three different experiments.

A23187 has a much steeper slope in adults than in old animals, a result that gives further support to the finding that the calcium buffering power of the mitochondrial matrix increases with age.

Effect of age on mitochondrial phosphate concentrations

In mitochondria incubated in the presence of P_i and ADP, the main component of the calcium buffering system is P_i. Thus, any increase in P_i results in a reduction of the mitochondrial free calcium concentration that leads to a lowering of the efflux rate [25,18]. Therefore, a possible cause for the difference in calcium buffering power of the mitochondrial matrix observed in old rats could involve endogenous P_i.

Table I shows that the concentration of endogenous P_i was significantly higher in old than adult mitochondria. Moreover, in phosphate-incubated mitochondria, the internal P_i concentrations were also higher in old animals.

Calcium is known to enhance phosphate uptake and ADP is required in order to prevent P_i and Ca efflux from mitochondria [18]. Accordingly, in the presence of calcium phosphate levels increase, even in the absence of added P_i, but the increase is more prominent in mitochondria from adults than

TABLE I

EFFECT OF AGE ON THE PHOSPHATE CONTENT OF MITOCHONDRIA

Brain mitochondria (1 m protein/ml) from 3- or 24-month-old rats were incubated as described in Methods during 3 min and the additions indicated. The results are means ± S.E.M. of three experiments.

Additions	3 months	24 months
None	4.39 ± 0.2	5.33 ± 0.16 [a]
25 nmol Ca^{2+}/ mg protein	11.40 ± 0.42	12.56 ± 0.35 [b]
25 nmol Ca^{2+}/mg protein, 0.2 mM ADP	11.45 ± 0.08	13.40 ± 0.36 [a]
0.8 mM P_i	19.55 ± 3.41	25.71 ± 2.24 [b]
0.8 mM P_i, 25 nmol Ca^{2+}/mg protein	29.67 ± 0.93	33.35 ± 1.46 [b]
0.8 mM P_i, 25 nmol Ca^{2+}/mg protein, 0.2 mM ADP	30.95 ± 1.23	39.52 ± 1.00 [a]

[a] $p < 0.0125$.
[a] $p < 0.05$.

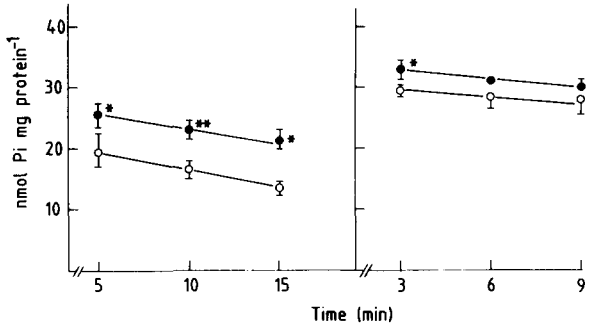

Fig. 4. Effect of age on P_i efflux from mitochondria. Brain mitochondria (1 mg protein/ml) from 3- (○) or 24-months-old rats (●) were incubated as described in Materials and Methods, in the presence of 0.8 mM P_i and in the presence (B) or absence (A) of 25 nmol Ca^{2+}/mg protein. P_i content of mitochondria was determined at the time intervals indicated (note the different time scales). The results are the mean ± S.E.M. of three experiments. * $P < 0.05$, ** $p < 0.125$.

in that from old animals (51% or 30% increase over values minus added Ca^{2+} in 3- and 24-month-old rats, respectively) (Table I). It is likely that the poor stimulating effect of calcium on P_i accumulation is due to the lower calcium uptake in mitochondria from old animals [8]. ADP addition does not modify any further the phosphate content of mitochondria from adults, but results in a new increase in P_i in mitochondria from old animals (Table I).

The increase in phosphate concentration found in mitochondria found old animals is probably not due to differences in P_i efflux, since the P_i efflux rates observed both in the absence or presence of calcium are not modified by age (fig. 4).

Discussion

We have shown that the Ca^{2+}-efflux rate decreases with age in rat brain mitochondria. The decrease in the rate of Ca^{2+} efflux could be due to variations in internal Ca^{2+} concentration or to antiporter derived effects. The experiments reported in this paper allow to distinguish between these possibilities.

Fig. 3 shows that the Ca^{2+}-buffering power of the mitochondrial matrix from old rats is higher than that of adults, indicating that the matrix-free Ca^{2+} concentrations at equivalent Ca^{2+} loads are higher in adult than in old animals. Since the

difference in matrix-free calcium concentrations is equivalent to the difference in H^+- and Na^+-dependent calcium efflux rates, our results suggest that carrier mediated effects are probably not involved. Hansford and Castro [28] have reported a similar decline in the calcium efflux activity of rat-heart mitochondria with age. It would be interesting to know whether changes in matrix calcium buffering power could be involved in this effect.

From the difference in phosphate content of mitochondria obtained from adult and old animals, it may be concluded that the increased calcium-buffering power of the mitochondrial matrix is associated with an increase in P_i content in mitochondria from old animals. This conclusion is supported by the following results. (a) The differences in calcium efflux rates become non-significant in the absence of added P_i (Fig. 1A), a condition in which the internal P_i concentrations are also similar in old- and adult-brain mitochondria (Table I, 2nd line). (b) The addition of ADP to mitochondria incubated in the absence of P_i leads to the appearance of age-dependent decrease in calcium efflux (Fig. 1B). This agrees with the increase in P_i content of old mitochondria observed under those conditions (Table I, 3rd line).

The cause of the higher phosphate content in brain mitochondria from old animals is unknown. Since we have found no differences in the P_i efflux rates with age (Fig. 4), the origin of P_i changes lies probably in the process of P_i accumulation. It is unlikely that the differences in P_i content are due exclusively to differences in the endogenous P_i of isolated mitochondria. Probably, the higher P_i content of mitochondria from old animals arises from a larger uptake of P_i with age, since the differences in P_i content after P_i addition are larger than the differences in endogenous P_i (Table I).

P_i uptake proceeds via $-OH^-$ exchange [29–31] and the steady-state distribution of P_i parallels that of ΔpH in nonrespiring mitochondria [30–32]. Since we have found no significant differences in the ΔpH of brain mitochondria from old animals using succinate as substrate in the absence or presence of P_i [33], changes in the driving force for P_i accumulation with age can be excluded.

In respiring mitochondria the P_i steady-state distribution deviates strongly from the predicted behaviour if P_i accumulation was only governed by ΔpH [34]. Greenbaum and Wilson [34] have suggested the existence of a mechanism to limit P_i accumulation in respiring mitochondria that would function to prevent a decrease in intramitochondrial free ADP. The prevention of P_i efflux from mitochondria by ADP [18] might operate through such a mechanism. It is possible that differences in endogenous ADP or in ADP sensitivity of P_i accumulation (see Table I) might be involved in the increase of P_i accumulation in old-rat-brain mitochondria.

The increase in the calcium buffering power of the mitochondrial matrix from 24-month-old rat brain might affect the metabolic response of this organelle to an increase in cytosolic calcium. Old-rat-brain mitochondria take up less calcium than those derived from adult animals [8]. In addition, the increase in free matrix calcium obtained after calcium uptake is markedly lower in the old animal. Thus, when exposed to similar changes in free cytosolic calcium, old-rat-brain mitochondria will respond with much smaller increases in matrix mitochondrial calcium than those derived from adults. Rat-brain oxoglutarate dehydrogenase is activated by calcium within the range of 10^{-6} to 10^{-5} M [35]. Oxoglutarate dehydrogenase, NAD-isocitrate dehydrogenase and pyruvate dehydrogenase from heart or liver mitochondria are activated by calcium within a similar concentration range and these enzymes can be activated within mitochondria by variations in extramitochondrial calcium concentrations (Refs. 9,36–41 and references therein). It is likely that dehydrogenases from brain mitochondria are subject to a similar regulation mechanism [10]. In that case, the activation of the dehydrogenases obtained after exposure to an equivalent change in cytosolic calcium could be substantially lower with age.

Hansford has pointed out [41] that the changes in pyruvate dehydrogenase activity brought about by cytosolic calcium concentrations in response to electrical stimulation [42,43] or training [44] could be important in neurotransmitter synthesis. According to that author [41] a consequence of increased activity of pyruvate dehydrogenase would be an increased mitochondrial acetyl-CoA/CoASH ratio that could be associated with increased rates of acetylcholine synthesis in re-

sponse to electrical activity [41]. Acetylcholine synthesis from ^{14}C-glucose decreases with age [1,5] and it has been suggested that this may result from differences in mitochondrial calcium concentration [41]. However, while other dehydrogenases might be involved, recent evidence could exclude that differences in the activation state of pyruvate dehydrogenase are responsible for this defect [45,46].

We have shown that the activity of NAD-isocitrate dehydrogenase decreases in rat-brain during ageing [15]. The failure to increase intramitochondrial calcium in old animals exposed to an increase in cytosolic calcium could contribute to the dramatic decrease of the flux through citrate-oxoglutarate found in old-rat brain [47].

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References

1 Gibson, G.E., Peterson, C. and Jenden, D.J. (1981) Science 213, 674–676
2 Sims, N.R., Manek, K.L., Bowen, D.M. and Davison, A.N. (1982) J. Neurochem. 38, 488–492
3 Allen, S.J., Benton, J.S., Goodhast, M.J., Haan, T.A., Sims, N.R., Smith, C.C.T., Spillane, J.A., Bowen, D.M. and Davison, A.N. (1983) J. Neurochem. 41, 256–265
4 Sims, N.R., Bowen, D.M., Allen, S.J., Smith, C.C.T., Neary, D., Thomas, D.J. and Davison, A.N. (1983) J. Neurochem. 40, 503–509
5 Gibson, G.E. and Peterson, C. (1981) J. Neurochem. 37, 978–984
6 Peterson, C. and Gibson, G.E. (1983) J. Biol. Chem. 258, 11482–11486
7 Leslie, S.W., Chandler, L.J., Barr, E.M. and Farrar, R.P. (1985) Brain Res. 329, 177–183
8 Vitórica, J. and Satrústegui, J. (1986) Brain Research 378, 36–48
9 Denton, R.M., McCormack, J.G. and Edgell, N.J. (1980) Biochem. J. 190, 107–111
10 Denton, R.M. and McCormack, J.G. (1980) FEBS Lett. 119, 1–8
11 Rapoport, S.I., Duara, R., London, E.D., Margolin, R.A., Schwartz, M., Cutler, N.R., Partanen, M. and Shinohara, N.L. (1983) in Ageing of the Brain (Samuel, D., ed.), pp. 111–121, Raven Press, New York
12 Gage, F.H., Kelly, P.A.T. and Bjorkluand, A. (1984) J. Neuroscience 4, 2856–2865
13 London, E.D., Naspar, S.M., Onata, M. and Rapoport S.I. (1981) J. neurochem. 37, 217–221
14 Patel, M.S. (1977) J. Gerontol. 32, 643–646
15 Vitórica, J., Andrés, A., Satrústegui, J. and Machado, A. (1981) Neurochem. Res. 6, 127–135
16 Smith, C.B. (1984) Trends Neurosci. 7, 203–208
17 Nicholls, D.G. (1978) Biochem. J. 170, 511–522
18 Vitórica, J. and Satrústegui, J. (1985) Biochem. J. 225, 41–49
19 Satrústegui, J. and Richter, C. (1984) Arch. Biochem. Biophys. 233, 736–740
20 Scarpa, A. (1979) Methods Enzymol. 56, 301–338
21 Lanzetta, P.A., Alvárez, L.J., Reinanch, P.S. and Candia, O.A. (1979) Arch. Biochem. 100, 95–97
22 Sokal, R.R. and Rohlf, S.J. (1981) in Biometry, pp. 461–505, Freeman, San Francisco
23 Siliprandi, D., Rugolo, M., Zoccarato, F., Toninello, A. and Siliprandi, N. (1979) Biochem. Biophys. Res. Commun. 88, 388–394
24 Toninello, A., Siliprandi, D. and Siliprandi, N. (1983) Biochem. Biophys. Res. Commun. 111, 792–797
25 Zoccarato, F. and Nicholls, D.G. (1982) Eur. J. Biochem. 127, 133–138
26 Nicholls, D.G. and Scott, I.D. (1980) Biochem. J. 186, 833–839
27 Goldstone, F.P., Duddridge, R.Y. and Crompton, M. (1983) Biochem. J. 210, 446–472
28 Hansford, R.G. and Castro, F. (1982) Mech. Ageing Devel. 19, 5–13
29 Chappel, J.B. and Crofts, A.R. (1966) in Regulation of Metabolic Processes in Mitochondria (Tager, J.M., Papa, J., Quagliariello, E. and Slater, E.C., eds.), pp. 293–314, Elsevier/North-Holland Biomedical Press, Amsterdam
30 McGivan, J.D. and Klingenberg, M. (1971) Eur. J. Biochem. 20, 392–399
31 Mitchell, P. and Moyle, J. (1969) Eur. J. Biochem. 9, 149–199
32 Papa, S., Lofrumento, N.E., Loglisci, M. and Quagliariello, E. (1966) Biochim. Biophys. Acta 189, 311–314
33 Vitórica, J., Clark, A., Machado, A. and Satrústegui, J. (1985) Mech. Ageing Devel. 29, 255–264
34 Greenbaum, N.L. and Wilson, D.F. (1985) J. Biol. Chem. 260, 873–879
35 Bernard, P.A. and Cockrell, R.S. (1984) Biochem. Biophys. Acta 766 (1984) 549–553
36 Denton, R.M., Richards, D.A. and Chin, J.G. (1978) Biochem. J. 176, 899–906
37 Denton, R.M., Randle, P.J. and Martin, B.R. (1972) Biochem. J. 128, 161–163
38 McCormack, J.G. (1985) FEBS Lett. 180, 259–264

39 McCormack, J.G. and Denton, R.M. (1984) Biochem. J. 218, 235–247
40 Hansford, R.C. and Castro, F. (1982) J. Bioeerg. Biomembranes 14, 361–376
41 Hansford, R.G. (1985) Rev. Physiol. Biochem. Pharmacol. 102, 1–72
42 Browning, M., Dunwiddie, T., Bennett, W., Gispen, W., Lynch, G. (1979) Science 203, 60–62
43 Browning, M., Bennet, W.F., Kelly, P., Lynch, G. (1981) Brain Res. 218, 255–266
44 Morgan, D.G. and Routtenberg, A. (1981) Science 214, 470–471
45 Deshmukh, D.R., Owen, D.E. and Patel, M.J. (1980) J. Neurochem. 34, 1219–1224
46 Ksiezak-Reding, H., Peterson, C. and Gibson, G.E. (1984) Mech. Ageing Devel. 26, 67–73
47 Zubairu, S., Hothershall, J.S., El-Hessan, A., McLean, P. and Greenbaum, A.L. (1983) J. Neurochem. 41, 76–83