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Differential Sensitivity of the Brain ATP-dependent and GTP-dependent Succinyl-CoA Synthetase to Vanadium Ions. Developmental Aspects

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Summary

We have recently found that both vanadate and vanadyl inhibit ATP-dependent succinyl-CoA synthetase (A-SCS) solubilized from the rat brain mitochondria. Aim of the present study was to estimate a proportion of A-SCS to G-SCS in adult and 5-day-old rat brain and their susceptibility to vanadium ions. The G-SCS to A-SCS ratio of 5-day-old brains was by 196 % higher than that in adults. This is in accordance with previous observation that G-SCS is high in tissues metabolizing ketone bodies. Both G-SCS and A-SCS differ in their susceptibility towards vanadium ions. A-SCS of adult brain was more sensitive to vanadate (IC 50 $1.6.10^{-5}$ mol.l^{-1}) than was G-SCS (IC 50 $6.2.10^{-5}$ mol.l^{-1}). On the contrary G-SCS was more sensitive to vanadyl (IC 50 $3.5.10^{-4}$ mol.l^{-1}) than was A-SCS (IC 50 $9.0.10^{-4}$ mol.l^{-1}). Also autophosphorylation of G-SCS a-subunit was more resistant to vanadate than A-SCS. In contrast to the adult SCS forms, almost equal susceptibility of A-SCS and G-SCS to vanadyl and vanadate was observed in infant brains. The results suggest some structural (functional) differences between two SCS forms in adults and also between infant and adult G-SCS.

Key words
Succinyl-CoA synthetase – Vanadate – Vanadyl – Brain – Development

Introduction

Originally, ATP-dependent succinyl-CoA synthetase (A-SCS) was assumed to occur only in bacteria and plants, whereas GTP-dependent succinyl-CoA synthetase (G-SCS) was believed to be confined to animal tissues (Bridger 1974). More recently, however, A-SCS has also been demonstrated in blowfly flight muscle and other animal tissues (Hamilton and Ottaway 1981, Hansford 1973, McClelan and Ottaway 1980, Weitzman *et al.* 1986). The ratio of A-SCS/G-SCS varies from tissue to tissue. Brain has a particularly high proportion of the A-SCS (Jenkins and Weitzman 1986). There is evidence that the two forms of SCS do not merely reflect the low specificity of the same enzyme but that they represent two different protein moieties, possessing different physical and other properties (Weitzman *et al.* 1986). A considerable rise in G-SCS in the brain under conditions of enhanced metabolism of ketone bodies, particularly during streptozotocin-induced diabetes, has been reported (Jenkins and Weitzman 1986). Two different roles for SCS have been suggested (Hamilton and Ottaway 1981, Jenkins and Weitzman 1986). The A-SCS would be primarily engaged in the tricarboxylic acid cycle catalysing the release of coenzyme A from succinyl-CoA. The G-SCS, thanks to the high GTP/GDP ratio (as in the brain mitochondria) catalyses the reverse reaction, formation of succinyl-CoA that can serve as a substrate for 3-oxoacid CoA transferase which in turn catalyses the formation of acetoacetyl-CoA ("active acetate"), an intermediate

Footnotes

Abbreviations: A-SCS, adenine nucleotide-dependent succinyl-CoA synthetase [succinic thiokinase; succinate:CoA ligase (ADP), EC. 6.2.1.5.]; G-SCS, guanine nucleotide-dependent succinyl-CoA synthetase [succinate:CoA ligase (GDP), EC. 6.2.1.4.]; TT, dithiothreitol EGTA, ethylenglycol-bis-(b-amino-ethylether)N,N tetraacetic acid.

in the ketone body metabolism. We have shown recently that both the vanadate anion (VO_3-, HVO_4^{2-}) and the vanadyl cation (VO^{2+}) inhibit A-SCS (Křivánek and Nováková 1991) and that the basic mechanism of this effect is attenuation of phosphorylation of the a-subunit of A-SCS (Křivánek and Nováková 1989b). In recent experiments, a comparison was made between the sensitivity of the two SCS forms to both vanadate and vanadyl ions. Since the 5-day-old rat brain is capable of utilizing ketone bodies (Drahota *et al.* 1965) the G-SCS/A-SCS ratio as well as the effect of vanadium ions on both SCS forms in the brain of 5-day-old rat was also tested.

Material and Method

Animals. The brain of adult (75 d) as well as of 5-day-old male hooded rats (Long-Evans strain) served as the source of mitochondria.

Chemicals. Meta-vanadate ($NaVO_3$) was from Aldrich-Chemie Steinheim, FRG), vanadyl sulfate was purchased from Janssen Chimica (Beerse, Belgium), ATP disodium salt, vanadate free and GTP was supplied by Boehringer (Mannheim, FRG), Coenzyme A, Na salt, and DTT were from Sigma Chemicals (St. Louis, MO, USA). Other chemicals were of grade purity (Lachema, Brno, Czechoslovakia). Before use, the vanadate solution was boiled to destroy dekavanadate present in the metavanadate preparation.

Preparation of mitochondria. The whole brain (except the cerebellum and brain stem) mitochondria were prepared by the procedure of Jones and Matus (1974). To obtain the total perikaryal plus synaptosomal mitochondria, the crude mitochondrial fraction was lysed in 5 mmol.l^{-1} Tris.HCl buffer, pH 8.1 and separated from myelin and membrane by combined flotation sedimentation–density gradient centrifugation procedure. The final mitochondrial pellet was washed with 4 mmol.l^{-1} imidazol buffer, pH 7.5 and stored as a suspension in a mixture of imidazole buffer-glycerol 1:1 (v/v) at -20 °C.

Solubilization of mitochondria. The mitochondrial suspension was mixed with the Lubrol-PX reagent under continuous stirring to a final concentration 0.5 % Lubrol in 0.25 mol.l^{-1} Tris.HCl, H 7.4, 9 % sucrose (Tsakiris 1984). After keeping the mixture at 4 °C overnight and spinning at 150 000 *g* for 1:30 h SCS activity was determined and related to the protein content of the Lubrol extracts.

Assay of SCS activity. The enzyme activity was determined by measuring the rate of change in absorption at 235 nm (Cha 1969). The Phillips UV/VIS spectrophotometer of series PU 8700 allowing work at high absorbance (up to 3.0 absorbance units) was used. The reaction mixture consisted of 50 mmol.l^{-1} Tris. succinate, pH 7.4., 10 mmol.l^{-1} $MgCl_2$, 0.1 mmol.l^{-1} ATP (GTP), 0.1 mmol.l^{-1} coenzyme A. After 3 min preincubation at 0 °C the reaction was started by adding an aliquot of the Lubrol extract. Lubrol by itself does not interfere under conditions used for SCS assay. The enzyme activity was expressed in nkat.mg prot.$^{-1}$ ± S.E.M. (kat = mol.s^{-1} in SI system).

Endogenous phosphorylation of mitochondrial proteins. The incubation medium of a total volume 50 ll consisted of 50 mol.l^{-1} Tris.HCl buffer, pH 7.4, 10 mmol.l^{-1} MgCl2, 2 mmol.l^{-1} GTA, 1mmol.l^{-1} DTT, mmol.l^{-1} 0.02 [gamma^{-33}P] GTP (4000 cpm per mol) and about 100 lg mitochondrial proteins. After 60 s preincubation with the labelled GTP phosphorylation was started by adding the mitochondrial suspension (10 ll) followed by 15 s incubation at 30 °C. The reaction was stopped by adding 50 ll of the Laemmli's sample solution and boiling for 3 min (Laemmli 1970). Quantification of the radioactivity of the a-subunit of SCS was performed by dissection of the 34.5 kDa band from stained, dry electrophoreograms and measuring its radioactivity by means of liquid scintillation (Křivánek and Nováková 1989a).

Proteins were determined by the method of Lowry *et al.* (1951). The bovine serum albumin was used as the standard.

Student's t-test was used for the statistical evaluation of the results.

Results

In accordance with the previously reported data (see Introduction) the activity of A-SCS in the adult brain markedly exceeds that of G-SCS (Table 1). The mean ratio of G-SCS to A-SCS was 0.26. In 5-day-old rats the activity of A-SCS was only about half of that in adults (1.69±0.06 v. 3.28±0.20), whereas the reverse was found for G-SCS (1.30±0.06 v. 0.84±0.05) and the ratio of G-SCS/A-SCS was 0.77 which is by 196 % higher than that in adults (Tab. 1, Fig. 1).

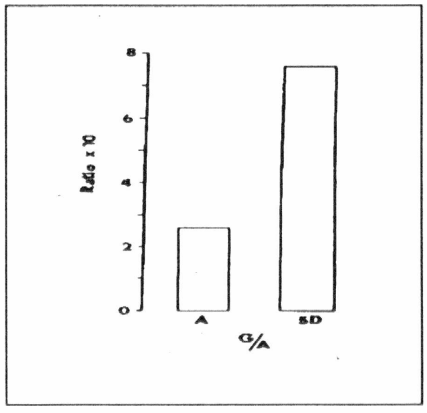

Fig. 1
The ratios of G-SCS/A-SCS activities (G/A) in adult (A) and 5-day-old (5D) rat brain mitochondria as calculated from the data in the Table 1.

Table 1
Effect of vanadate (10^{-5} M) on ATP- and GTP-dependent succinyl-CoA synthetase solubilized from rat brain mitochondria. Values are means of units ± S.E.M.

Age	ATP-SCS			GTP-SCS		
	Control	Vanadate	P	Control	Vanadate	P
Adult	3.28 ± 0.20 (n = 10)	1.77 ± 0.16 (n = 10)	< 0.003	0.84 ± 0.05 (n = 6)	0.63 ± 0.05 (n = 6)	< 0.05
5-day-old	1.69 ± 0.06 (n = 10)	0.86 ± 0.04 (n = 10)	< 0.003	1.30 ± 0.06 (n = 10)	0.72 ± 0.04 (n = 10)	< 0.003

Table 2
Effect of vanadyl (10^{-5} M) on ATP-dependent and GTP-dependent succinyl-CoA synthetase solubilized from rat brain mitochondria. Values are means of units ± S.E.M.

Age	ATP-SCS			GTP-SCS		
	Control	Vanadyl	P	Control	Vanadyl	P
Adult	3.13 ± 0.19 (n = 9)	3.10 ± 0.19 (n = 9)	N.S.	0.74 ± 0.02 (n = 9)	0.59 ± 0.03 (n = 9)	< 0.003
5-day-old	1.76 ± 0.11 (n = 6)	1.49 ± 0.10 (n = 6)	N.S.	1.25 ± 0.09 (n = 6)	1.07 ± 0.08 (n = 6)	N.S.

The difference in activity of the two SCS species recovered from adult and infant mitochondria may merely reflect the difference in the solubility of A-SCS and G-SCS from mitochondria of different age. To see whether some qualitative differences between G-SCS and A-SCS at the two ages could be found, the response to vanadium ions of the two SCS species solubilized from infant and adult brain mitochondria was tested. As is shown in Tab. 1, vanadate at the concentration 10^{-5} mol.l^{-1} inhibited the activity of A-SCS in the brain of both ages to the same extent (47 % and 49 % for adult and infant rats, respectively). However, G-SCS in the adult brain was more resistant towards the effect of vanadate (only 23 % inhibition) as compared with that of 45 % in 5-day-old brains ($P < 0.003$). Thus, not only a difference in the sensitivity to vanadate of the separate species of SCS but also a different sensitivity between the response of G-SCS was observed at different ages. Vanadyl (10^{-5} mol.l^{-1}) exerted a more pronounced inhibitory effect on G-SCS than that on A-SCS (Tab. 2). Vanadyl at this concentration is quite ineffective toward A-SCS while G-SCS was inhibited by 20 % ($P < 0.003$). In 5-day-old mitochondria, no difference between A-SCS and G-SCS in their susceptibility to vanadate has been observed (Table 1 and 2). Dose-dependent curves (Fig. 2 and 3) demonstrate the difference in SCS responses to vanadium ions even more clearly. Vanadyl at the concentration 10^{-4} mol.l^{-1} inhibited adult G-SCS by 27 %, whereas A-SCS was inhibited by only 14 %.

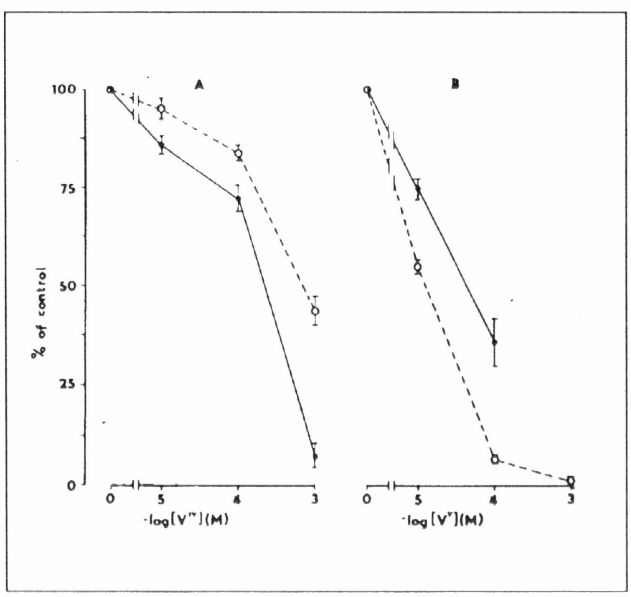

Fig. 2
Dose-dependence curves for the vanadium ions effects on the A-SCS (open circles) and G-SCS (black circles) of the brain mitochondria from adult rats. Each point represents the mean of at least 9 measurements ± S.E.M. A – effect of vanadyl; B – effect of vanadate. P values for the differences between A-SCS and G-SCS inhibitions: At 10^{-5} mol.l^{-1}, $P < 0.05$; at 10^{-4} mol.l^{-1}, $P < 0.01$; at 10^{-3} mol.l^{-1}, $P < 0.003$. B – effect of vanadate. P values: at 10^{-5} mol.l^{-1}, $P < 0.003$; at 10^{-4} mol.l^{-1}, $P < 0.01$.

At the concentration 10^{-3} mol.l^{-1} the respective inhibitions were 93 % and 56 %. On the other hand, vanadate exerted a more pronounced effect on the A-SCS which was inhibited by 43 %, 93 % and 100 % at 10^{-5}, 10^{-4} and 10^{-3} mol.l^{-1}, respectively, while the G-SCS was inhibited by only 25 % and 67 % at 10^{-5} and 10^{-4} mol.l^{-1} vanadate. The two forms of SCS from the brain mitochondria of 5-day-old rats did not differ in their response to vanadyl or vanadate. This is particularly well expressed for vanadate which inhibited both A-SCS and G-SCS to the same extent. Vanadyl at 10^{-4} mol.l^{-1} concentration inhibited G-SCS by 29 % and A-SCS by 26 %. From these curves it is possible to estimate roughly the IC50 values. For vanadyl inhibition of adult G-SCS it was $3.5.10^{-4}$ mol.l^{-1} while for A-SCS $9.1.10^{-4}$ mol.l^{-1}. Vanadate, however, exerted more pronounced effect on A-SCS, with IC50 $1.5.10^{-5}$ mol.l^{-1} as compared to $6.2.10^{-5}$ mol.l^{-1} for G-SCS.

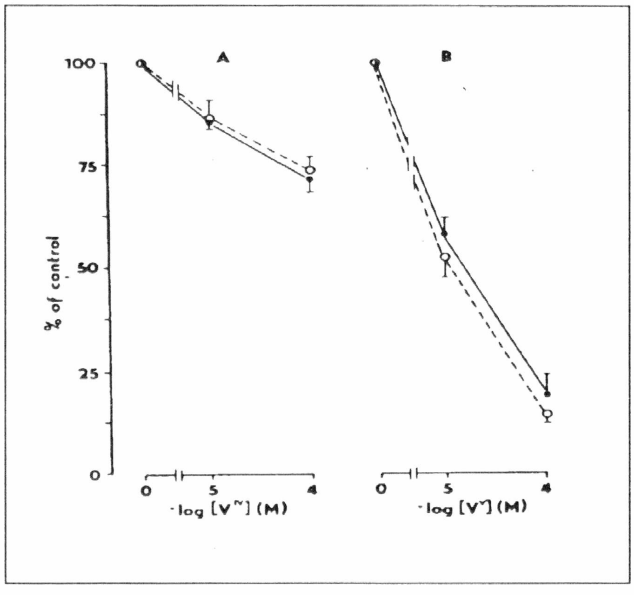

Fig. 3

Dose-dependence curves for the effects of vanadium ions on the A-SCS (open circles) and G-SCS (black circles) of the brain mitochondria from 5-day-old rats. Each point represents the mean of at least 6 measurements ± S.E.M. A – effect of vanadyl; B – effect of vanadate.

Vanadate has been shown to inhibit phosphorylation of the a-subunit of A-SCS (Křivánek and Nováková 1989 a,b). To see whether also phosphorylation of the a-subunit of G-SCS from the brain will differ from that of A-SCS, we tested the effect of vanadate on endogenous phosphorylation of the mitochondrial proteins using GTP as a phosphate donor. Fig. 4 shows the vanadate concentration dependent inhibitory curve of phosphorylation of the a-subunit of G-SCS as compared with that demonstrated previously for A-SC (Křivánek and Nováková 1989 a,b). It can be seen that phosphorylation of G-SCS is indeed less susceptible to the inhibitory action of vanadate. At 10^{-5} mol.l^{-1} vanadate concentration no inhibition of phosphorylation of the a-subunit of G-SCS was observed while that of A-SCS was inhibited by 25 %. At 10^{-4} mol.l^{-1} vanadate phosphorylation 63 % and 25 % inhibition was found for A-SCS and G-SCS respectively.

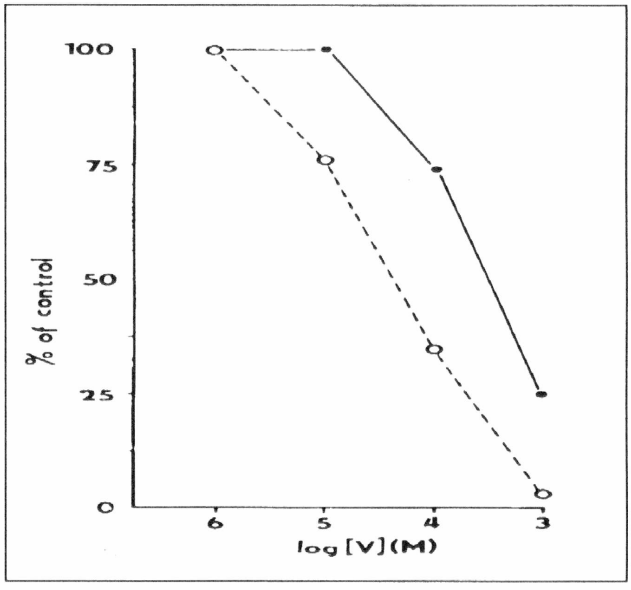

Fig. 4

Dose-dependence curves for the vanadate effect on phosphorylation of the -subunit of the A-SCS (open circles) and that of G-SCS (black circles) of the brain mitochondria from adult brain. The values for the A-SCS are from the paper by Křivánek and Nováková (1989b).

Discussion

The data presented in this communication show that the ratio of G-SCS/A-SCS is considerably higher in the brain mitochondria of 5-day-old rats than in those of adult rats. Since the brain of infant rats is able to utilize ketone bodies as oxidizable substrate (Drahota *et al.* 1956), these results could be considered as further support for the assumption that GTP- dependent succinyl-CoA synthetase is primarily engaged in metabolism of ketone bodies providing succinyl-CoA for 3-oxoacid CoA transferase, catalysing formation of acetoacetyl-CoA a key intermediate in the ketone body metabolism (Hamilton and Ottaway 1981, Jenkins and Weitzman 1986). Mitochondrial density as well as the solubility of mitochondrial enzymes may differ during development. Thus the different activities recovered in the Lubrol extracts may reflect merely differential solubility of the two SCS forms from mitochondria of different age. To estimate such a partition between mitochondrial and non-mitochondrial compartments is difficult because the enzyme is tightly bound on these particles. Its activity in intact mitochondria is very low

(Hamilton and Ottaway 1981). Although the difference in G-SCS/A-SCS ratio in infant and adult rat brain observed in the present experiments is in good correlation with previously published data (Jenkins and Weitzman 1986), the contribution of differential solubilization cannot be excluded. However, the experiments also demonstrate a differential susceptibility to vanadium ions suggesting a difference in some structural (functional) properties of at least those fractions of two SCS forms that were solubilized by Lubrol. The results suggest that not only A- and G-forms of SCS represent distinct molecular species as has already been claimed (Weitzman *et al.* 1986) but that G-SCS from infant and adult brain may also differ.

Less markedly expressed vanadate inhibition of the adult G-SCS may be reasonably explained by lower sensitivity of its a-subunit phosphorylation to vanadate as compared with that of A-SCS (Fig. 4). The possible participation of other yet unknown mechanism(s) cannot be excluded, however. Although vanadyl has been suggested to be the biologically active form of vanadium in living cells (Cantley and Aisen 1979, Macara *et al.* 1980), its biochemical effects have been studied to a much lesser extent than those of vanadate. In most studies, vanadyl has been shown to be much less biochemically active than vanadate (see Křivánek and Nováková 1988 for references). In a recent study, vanadyl revealed a greater effect on the adult G-SCS as compared to that on the adult A-SCS. The mechanism of this difference is not known. Even the basic mechanism of the effect of vanadium ions on SCS is not yet definitely established. The phosphoenzyme is obvious intermediate in the SCS catalytic mechanism so that inhibition of phosphorylation of the a-subunit is an essential step in the inhibition of SCS activity. However, interaction of phosphate residing on the a-subunit with succinate which is bound to the b-subunit as well as some other factors may contribute to the gross catalytic mechanism (Bridger 1974). The dependence of vanadate inhibition of SCS on the succinate concentration (unpublished results) fit in with this assumption. The complexity of the vanadium ions effect on SCS rules out making any (speculative) conclusion about the details of the mechanism of the effect of vanadium ions on SCS. It is also difficult to estimate what features of the SCS forms are responsible for the different susceptibility to vanadium ions. The results, however, do provide further evidence that the two SCS forms represent two distinct molecular moieties (Weitzman *et al.* 1986) that in turn may also be distinguishable by their reaction to vanadium ions.

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Reprint Requests

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