ATPases of the Cat Carotid Body and of the Neighbouring Ganglia

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The average ATPase activity in homogenates of the cat carotid body is found to be 20 nmol P_i liberated per minute per mg fresh tissue weight at 25 °C. ATPase activity in the nodose ganglion and the superior cervical ganglion is found in the same range. Most of the activity remains in the supernatant after removal of the mitochondria by centrifugation. The activity is inhibited by ouabain only marginally (ganglia) or not at all (carotid body). In all these organs up to 80% of the activity is seen in the absence of Mg^{2+} and the presence of increasing concentrations of Ca^{2+}.

In the carotid body of the cat, changes in Ca^{2+} activity are observed during neural activity using ion selective electrodes. During hypoxia of the superfused carotid body, a decrease of Ca^{2+} activity was found in the extracellular space, whereas Type I cells of the carotid body held in primary culture showed an increase in intracellular Ca^{2+} activity (Acker *et al.* [1]).

Other authors reported a release of catecholamines upon chemoreception of the carotid body. Though the observations differ in detail, catecholamines seem to play a role in chemoreception [2, 3].

These two lines of observations may have a common root, as electron microscopical studies suggest an influence of Ca^{2+} on catecholamine release [4, 5].

A stimulation of Na^+-K^+-dependent ATPases by catecholamines has been reported for nervous tissue [6–8]. Several authors found a release of neurotransmitters during an inhibition of the Na^+-K^+-dependent ATPase by Ca^{2+} [9, 10].

Because of these reports, we became interested, whether ATPases are present in the carotid body and whether they can be influenced by Ca^{2+}. If present, such ATPases might be linked directly to Ca^{2+} transport and indirectly to the release of neurotransmitters. In the literature, only histochemical and electron microscopical observations on ATPases in the carotid body are found [11, 12]. Biochemically, Bonting *et al.* measured ATPase activity in the superior cervical ganglion of the cat and found it extremely low [13].

In the present study, we report measurements of ATPase activity in the carotid body and in its neighbouring ganglia in the presence of several ions, and of inhibitors. By these measurements we wanted to compare the ATPases of these organs with other enzymes of this kind which are known to be involved in ion transport and/or in the release of neurotransmitters in other organs.

Methods

The carotid body and the other tissues used in this study were excised from cats anesthetized with Nembutal. All subsequent steps were done at 4 °C. The tissues were cut into small pieces and homogenized, suspended in 0.01% Triton X100 or in 250 mM sucrose to a concentration of 2–30 mg tissue/ml, and homogenized by a passage through a Potter-Elvehjem homogenizer.

The activity of ATPase was determined in the homogenate directly or after fractionation by differential centrifugation.

This fractionation was done by centrifugation of the homogenate and the subsequent supernatants for 10 min at $480 \times g$, for 10 min at $12\,000 \times g$ and for one hour at $100\,000 \times g$. Activities were determined both in the supernatant and in the sediment after resuspension.

Prior to the determinations, some of the fractions were adjusted to 0.01% Triton X100. No significant differences in the ATPase activity were found in the

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presence and in the absence of the detergent. Therefore, Triton was omitted from the later experiments, and activities were averaged using both Triton-containing and Triton-free samples. If possible, determinations were done immediately following the excision of the tissues. In some cases, homogenates or fractions were stored at −20 °C for a period up to several days, and no decrease in activity in excess of 10% was observed.

For the determination, the samples were diluted to a concentration of 5−20 µg/0.2 ml sample size. Incubations were done either at 25 °C or at 37 °C.

The duration of the incubation was varied between 10 and 30 min. Most of the incubations were done for 20 min at 25 °C. The reaction was terminated by the addition of sulfuric acid, and the inorganic phosphate liberated was determined by the method of Martin and Doty [14]. Blanks were done either by using boiled samples, or by incubating the incubation mix without protein, the latter being added immediately before the termination of the reaction. The standard incubation mix was 50 mM Tris acetate, 2.5 mM ATP, 2.5 mM $MgCl_2$, 100 mM NaCl, 15 mM KCl, pH 7.5. In some cases, L-histidine was added to 25 mM. For the determination of the Na^+-K^+-ATPase, KCl was replaced by 0.2 mM ouabain, or both NaCl and KCl were omitted. In other experiments, Mg^{2+} was replaced by Ca^{2+}, or the effect of other bivalent cations or other chemicals was determined in the presence of either Mg^{2+} or Ca^{2+}, as mentioned in the Results section.

The activity of ATPase is given as nmole P_i (=inorganic phosphate) liberated per minute per mg fresh weight (or equivalent amount of fractions). In the case of the carotid body, weighing was not practical in each experiment. Here, the activities are given as nmole P_i liberated per minute per organ. The weight of a cat carotid body was assumed to be 0.4 mg (average of 54 cats [15]).

In some cases, the activity was expressed per mg protein. Protein determinations were done by the method of Lowry *et al.* [16].

Results

Dependency of ATPase activities on sample size, temperature, reaction time and pH

At 25 °C, the phosphate liberated was proportional to the amount of the extract between 10 and 300 µg fresh tissue weight for all organs investigated. The reaction time was 10 min for the larger and 20 min for the smaller amounts of tissue. Fig. 1 shows the activity of the carotid body homogenate incubated for 20 min. Incubation at 37 °C resulted in an average increase in activity of 50% (data not

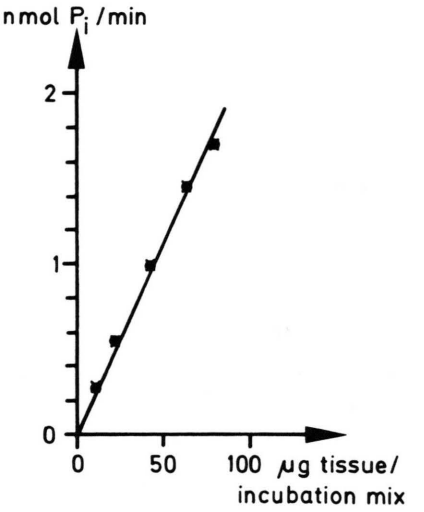

Fig. 1. ATPase activity in the homogenate of the carotid body. Incubation time: 20 min, $t = 25$ °C. Incubation mix: 50 mM Tris acetat, 2.5 mM ATP, 2.5 mM $MgCl_2$, 100 mM NaCl, 15 mM KCl, pH 7.5.

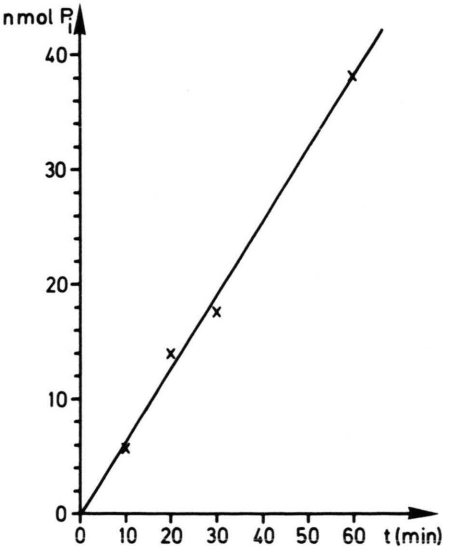

Fig. 2. Time dependency of the ATPase reaction in homogenates of the carotid body. Incubation mix and temperature: see legend to Fig. 1.

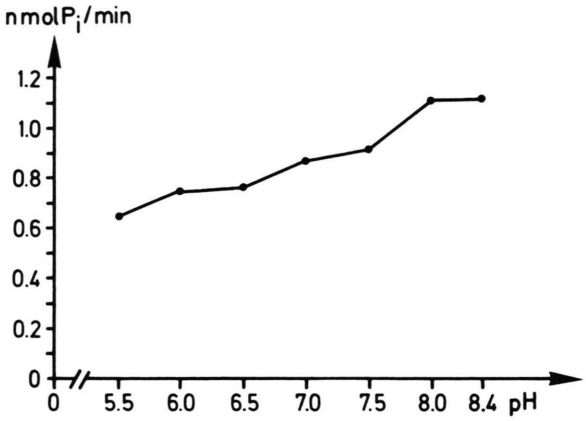

Fig. 3. pH dependency of the ATPase reaction in homogenates of the carotid body. Incubation time: 20 min. Incubation mix and temperature: see legend to Fig. 1.

shown). The liberation of phosphate was linear between 10 and 60 min, as long as the amount liberated did not exceed 30 nmol (Fig. 2).

Fig. 3 shows the influence of pH on the activity of carotid body homogenates. Here as well as in the extracts obtained from the other tissues investigated, the activity increased from pH 5.5 to 8.2. No maximum was observed at pH 7.5, the pH used in the standard incubation mix.

The ATPase activity of homogenates in standard medium, K^+ omitted and 0.2 mM ouabain added, or after substitution of Mg^{2+} by other bivalent cations

Table I shows standard activities for the three organs. They are found between 14 and 20 nmol P_i/mg fresh weight. No significant differences between the organs are seen.

Addition of ouabain and omission of K^+ resulted in activities of 96% (carotid body), 90% (superior cervical ganglion) and 94% (nodose ganglion) as compared to standard incubations. The reduction was significant for the superior cervical ganglion ($p = 0.01$) and the nodose ganglion ($p = 0.05$), when evaluated by Student's *t*-test for paired comparison. No significant reduction was seen for the carotid body.

Replacement of Mg^{2+} by 5 mM Ca^{2+} resulted in an average reduction to 81% for the glomus, to 79% for the superior cervical ganglion, and to 79% for the nodose ganglion. No single experiment showed 100% of the standard activity in the presence of Ca^{2+}.

Lower activities only were seen, when Mg^{2+} was replaced by 2 mM Mn^{2+}, 2 mM Co^{2+} or 2 mM Zn^{2+}. The average activity in the presence of Mn^{2+} was 60% (3 experiments). Zn^{2+} led to a reduction to 20%, and was thus the least effective replacement for

Table I. ATPase activity in homogenates ($t = 25$ °C; pH = 7.5)

Organ	nmol P_i/(mg fresh weight × min.)					
	Standard incubation mix	0.2 mM Ouabain	5 mM Ca^{2+}	2 mM Mn^{2+}	2 mM Co^{2+}	2 mM Zn^{2+}
	$\bar{x}$ s					
Carotid body[a]	20.5±13.7 (36)					
	21.0±10.3 (16)	20.2±9.7 (16)				
	22.1±12.5 (13)		18.0±10.5 (13)			
	26.9 (3)			16.2 (3)	10.4 (3)	3.6 (3)
Superior cervical ganglion	17.9± 9.4 (41)					
	16.8± 7.1 (25)	15.2±6.4 (25)				
	20.1±12.1 (14)		15.9±10.6 (14)			
	20.9 (3)			14.6 (3)	10.9 (3)	4.3 (3)
Nodose ganglion	14.8± 7.2 (28)					
	16.0± 8.4 (14)	15.1±8.2 (14)				
	19.3± 7.6 (7)		15.3± 6.5 (7)			
	18.0 (2)			10.5 (2)	9.1 (2)	4.3 (2)

$\bar{x}$ = mean value; s = standard deviation; brackets = number of experiments.
[a] The fresh weight of one carotid body was assumed to be 0.4 mg [15]. Standard incubation mix: 2.5 mM $MgCl_2$, 2.5 mM ATP, 100 mM NaCl, 15 mM KCl. Ouabain incubation mix: 15 mM KCl was omitted and 0.2 mM Ouabain added. Ca^{2+} incubation mix: 2.5 mM $MgCl_2$ was replaced by 5 mM $CaCl_2$. Mn^{2+}, Co^{2+}, Zn^{2+} incubation mix: Mg^{2+} was replaced by 2 mM of the indicated ion. The values in the left column are to be compared with the values in the same row of the other columns.

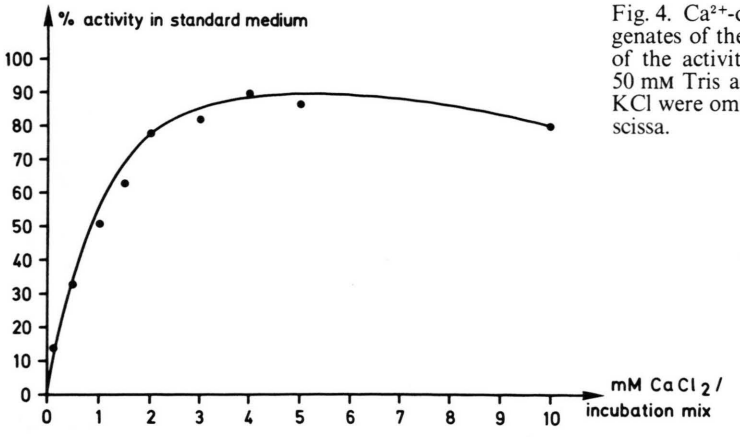

Fig. 4. Ca^{2+}-dependency of the ATPase activity in homogenates of the carotid body. The activity is expressed in % of the activity in the standard medium. Incubation mix: 50 mM Tris acetat, 2.5 mM ATP, pH 7.5 $MgCl_2$, NaCl and KCl were omitted. $CaCl_2$ was added as indicated in the abscissa.

Mg^{2+}. Fig. 4 shows a concentration dependence for Ca^{2+} in a homogenate of the carotid body. In the absence of Ca^{2+}, blanks are obtained, while in the presence of 2 mM Ca^{2+} nearly maximal values are obtained. No further increase is seen up to a Ca^{2+} concentration of 10 mM.

In Table II, the influence of various additions to either standard medium or standard medium $-Mg^{2+}$ $+Ca^{2+}$ is given. Though in some experiments the concentrations of ATP, Mg^{2+} or Ca^{2+} are lower than in Table I, the values obtained do not differ significantly from those obtained under standard conditions.

The experiments listed in Table II which contained Ca^{2+} in the incubation mix cannot be compared directly to those containing Mg^{2+}, as the homogenates came from different animals.

Both in the presence of Mg^{2+} and of Ca^{2+}, GTP, UTP, and CTP are very suitable substrates for the enzyme liberating inorganic phosphate.

ADP in the presence of Ca^{2+} yielded 35% of the standard activity obtained with ATP.

As Table III shows most of the activity is found in the 12000×*g* supernatant. Much of the activity remains in the supernatant even after 1 h at 100000×*g*, though the activity per mg protein is then higher in the sediment.

Sucrose homogenates are virtually free of mitochondria after 10 min at 12000×*g* as was confirmed by electron microscopy, kindly performed by D. Schäfer. In order to further test that the activity observed in the homogenates is due to an ATPase not of mitochondrial origin, these supernatants were used for experiments, in which K^+ was replaced by ouabain or Mg^{2+} by Ca^{2+}. The average activities measured in these experiments are shown in Table IV. Many of the supernatants listed in this table were obtained from homogenates that came from other animals than the unfractionated homogenates. This explains that the numerical values are so similar. If a supernatant is obtained from a homogenate that is also tested unfractionated, the supernatants always yield lower activities.

The results obtained were not different from those obtained with homogenates: Ouabain reduced the activity in the two ganglia only slightly in the presence of Mg^{2+}, and not at all in the presence of Ca^{2+} (in all three organs). The other three nucleoside

Table II. P_i liberation in homogenates by application of different substrates in presence of Mg^{2+} of Ca^{2+} ($t = 25$ °C, pH = 7.5)

Organ	Ion	nmol P_i/(mg fresh weight×min)	Activity in percent of the ATP cleavage			
		ATP	ADP	GTP	UTP	CTP
Carotid body	Mg	25.3 (4)	18	89	81	80
	Ca	11.5 (5)	43	104	104	110
Superior cervical ganglion	Mg	24.0 (4)	20	86	73	73
	Ca	12.6 (4)	35	93	98	95
Nodose ganglion	Mg	23.8 (4)	19	86	73	75
	Ca	17.3 (3)	27	81	84	91

Incubation mix: 1.5 mM Mg^{2+} or 1.5 mM Ca^{2+} (in some experiments 5 mM Ca^{2+}), 100 mM NaCl, 15 mM KCl, 50 mM Tris-acetat, all substrates 1 mM.

Table III. ATPase activity of homogenates and fractions after centrifugation ($t = 25$ °C, pH = 7,5).

Organ	Homogenate		10 min Centrifugation						1 h Centrifugation			
			Homogenate at 480×g		480×g Supernatant resp. homogenate at 12 000×g				12 000×g Supernatant at 100 000×g			
	nmol P_i/(mg · min)		Sediment nmol P_i/(mg · min)		Sediment nmol P_i/(mg · min)		Supernatant nmol P_i/(mg · min)		Sediment nmol P_i/(mg · min)		Supernatant nmol P_i/(mg · min)	
	Fresh weight	Protein	Fresh weight	Protein	Fresh weight	Protein	Fresh weight	Protein	Fresh weight	Protein	Fresh weight	Protein
Carotid body	74.0 (3) 42.6 (12)		1.8 (3)		0.8 (3)		67.0 (3) 35.3 (12)		25.9 (3)		36.8 (3)	
Superior cervical ganglion	35.8 (4) 53.7 (2) 32.2 (5) 34.1 (10)	 927.3 (2) 489.7 (5)	1.75 (4) 2.1 (2)	 180.4 (2)	0.83 (4) 0.58 (2)	 351.0 (2)	32.8 (4) 52.5 (2) 34.7 (5) 28.2 (10)	 1069.5 (2) 684.6 (5)	17.0 (4) 26.0 (2)	 2476.9 (2)	13.3 (4) 19.8 (2)	 588.6 (2)
Nodose ganglion	61.9 (3) 84.6 (2) 47.3 (5) 46.9 (5)	 1101.4 (2) 603.4 (5)	0.76 (3) 0.80 (2)	 96.0 (2)	0.95 (3) 1.28 (2)	 240.2 (2)	54.9 (3) 76.3 (2) 40.9 (5) 46.0 (5)	 1111.6 (2) 803.1 (5)	27.9 (3) 39.4 (2)	 2669.9 (2)	21.9 (3) 29.4 (2)	 642.1 (2)

Incubation mix: Table I. The values in the left columns are to be compared with the values in the same row of the other columns.

Table IV. ATPase activity in *mitochondria-free* supernatant of homogenates (t = 25 °C, pH = 7.5).

Organ	Ion	nmol P_i/ (mg fresh weight × min) ATP	Percent of the ATP cleavage				
			+ Ouabain	ADP	GTP	UTP	CTP
Carotid body	Mg	20.6 ± 13.3 (16)					
		6.1 (2)	110				
		21.5 (4)		18	81	73	67
	Ca	18.0 ± 12.4 (16)					
		4.7 (2)	100				
		21.0 (1)		18	76	106	75
Superior cervical ganglion	Mg	18.1 ± 13.1 (19)					
		16.3 ± 4.9 (6)	94				
		23.2 (4)		13	87	75	68
	Ca	14.6 ± 10.2 (19)					
		13.7 ± 5.2 (6)	103				
		14.2 (2)		17	76	95	99
Nodose ganglion	Mg	12.3 ± 2.4 (6)					
		18.8 ± 5.5 (5)	96				
		17.4 ± 7.4 (6)		15	74	74	70
	Ca	9.5 ± 2.2 (6)					
		15.0 ± 4.6 (5)	103				
		10.6 (4)		32	81	106	113

Incubation mix with Mg^{2+} or Ca^{2+}: Table I. If ATP is replaced by other substrates incubation mix: Table II. The values in the left column are to be compared with the values in the same row of the other columns.

triphosphates used instead of ATP were good substrates for the enzyme.

Antimycin (10^{-4} M), oligomycin (15.5 µg/ml), cysteine (10^{-4} M) and D 600 (100 µg/ml) gave reductions in activity lower than 10% for all three organs. This is in marked contrast to the strong inhibition of mitochondrial ATPases by antimycin and oligomycin, or to the stimulation of intestinal Ca^{2+} ATPase by cysteine [19]. NaN_3 at a concentration of 5×10^{-3} M reduced the ATPase activity by 30%.

Discussion

In the carotid body, the ATPase activity per mg fresh tissue is similar to that found in the neighbouring ganglia.

Though the standard error of the measurements was large (Table I), even the lowest values found were higher by an order of magnitude than those reported by Bonting [13] for the cervical ganglion.

The highest activities were found in ca. 6 weeks old animals that had lived under optimal conditions and were used immediately after the separation from their mothers. However, even adult animals, whose organs were homogenised and ATPases determined according to the method of Bonting, yielded results within the range of measurements of all of our experiments. We do not know the reason for this discrepancy.

The distribution of activities in the fractions obtained by centrifugation was virtually identical for the three organs investigated. The highest fraction of the total activity was found in the supernatant, from which both coarse particles and mitochondria had been removed.

The striking similarity of the ATPases from the three organs is exhibited by their response to bivalent cations in the absence of Mg^{2+}, and by their action on nucleoside triphosphates other than ATP.

In the case of ouabain inhibition, the two ganglia showed a slight reduction of activity (6 and 10% for the nodose and cervical ganglion, respectively) which is significant by Student's t-test for paired values. No significant reduction is seen in the case of the carotid body. As ouabain is known to inactivate the Na^+- and K^+-dependent ATPases, it must be concluded that the fraction of this ATPase among the ATPases tested is very small, or, in the case of the carotid body, even below the level of detection.

This small fraction of ouabain-sensitive ATPase seems to have an absolute Mg^{2+} requirement, because the activity remaining after replacement of Mg^{2+} by Ca^{2+} showed no further decrease in the presence of ouabain.

In parallel experiments with rat brain homogenates (13 experiments, data not shown), ouabain replacing K^+ reduced the activity to 34%. These experiments excluded the possibility that the failure to find an ouabain response in our experiments was due to technical reasons. Also, no stimulation of the ATPases obtained from the carotid body and the two ganglia was obtained after the addition of $10^{-6}-10^{-5}$ M noradrenalin (data not shown). This addition has been reported, however, to stimulate the $K^+ - Na^+$-dependent ATPases from rat brain cortex homogenates [4]. We conclude that our ATPase(s) is not of the $Na^+ - K^+$-dependent type.

The fractionation experiments and the lack of response to various typical inhibitors further exclude that our enzyme is of mitochondrial origin.

Notwithstanding the possibility of the formation of loose complexes with structured elements of the cells, the majority of the enzyme is found in soluble form and may be a soluble component of the cytoplasm.

In the presence of Mg^{2+} in optimal concentration, Ca^{2+} had no detectable effect on the activity (no difference in either 10^{-4} M Ca^{2+} or 10^{-4} M EGTA, data not shown). In the absence of Mg^{2+}, however, a high activity could be sustained by Ca^{2+} alone.

If the ATPase described here is compared to ATPases described in the literature [17–20] and to those seen in our unpublished experiments with homogenates from muscle, liver, adrenal gland and rat brain, distinct differences are observed. Even if we limit our comparison to those ATPases that are activated by Ca^{2+} in the absence of Mg^{2+}, the similarities are limited [21, 22].

An ATPase found in the microsomal fraction of the frog sciatic nerve by Edström [21] resembles our ATPase with regard to Mg^{2+} and Ca^{2+} stimulation, but differs with regard to replacement of ATP by other nucleoside triphosphates.

In Edström's experiments, the P_i-liberating activity was stimulated by a factor of 3, when ATP was replaced by CTP in the presence of Ca^{2+}. In our experiments, the activity found with CTP instead of ATP in the presence of Ca^{2+} was at most 10% higher (in the carotid body), or not increased (in the ganglion) (5 experiments).

In conclusion, the ATPases found in the carotid body and its two neighbouring ganglia cannot be distinguished from each other. They are clearly different, however, from other ATPases, both from the nerve cells of the rat brain and from other tissues.

Whether the high number of free nerve endings in the carotid body [23] and the ganglia is the source of this ATPase, and whether it has a unique physiological role, cannot be decided by the present experiments.

Taugner has described an ATPase, the activity of which is linked to catecholamine uptake by storage vesicles in the adrenal medulla. This ATPase is structure-bound, has a pH maximum at pH 7.4 and thus also seems to be different from our ATPase. However, the enzyme responsible for catecholamine transport in adrenal medulla may constitute only a minor fraction of the total ATPases in that organ, and Ca^{2+} dependency in the absence of Mg^{2+} has not been determined [24]. Because catecholamine transport is a function related to those discussed for chemoreception in the carotid body, a more detailed comparison of these two activities is necessary to decide whether there are similarities or not.

Acknowledgement

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