Effects of 2-Deoxy-D-Glucose on In Vitro Cat Carotid Body

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The process of chemosensory transduction in the arterial chemoreceptors is not well understood. According to the metabolic hypothesis of chemoreception, a decrease in arterial \( pO_2 \) will produce a decrease in the adenosine triphosphate (ATP) content in the chemosensory type I cells, leading to release of a neurotransmitter and increased sensory neural activity. There is a paucity of direct experimental support for this hypothesis, and in the present work, we have tested the postulates of the metabolic hypothesis in an in vitro preparation of cat carotid body using 2-deoxy-D-glucose as an ATP-depleting agent. This preparation, when superfused with Tyrode containing 5 mM \( Na^+ \)-pyruvate instead of glucose, responds normally to hypoxia, low pH and acetylcholine, and maintains normal ATP levels. Under these conditions, 2-deoxy-D-glucose is a chemostimulant, i.e. electrical activity in the carotid sinus nerve is increased, with a threshold concentration of 0.25 mM and a maximum response at about 2–4 mM. These concentrations of 2-deoxyglucose evoke a dose-dependent release of \([H]\)dopamine (synthesized from \([H]\)tyrosine) from the carotid bodies which parallels the electrical activity. The 2-deoxy-D-glucose-evoked release and electrical activity is dependent on the presence of extracellular Ca\(^{2+}\). These same concentrations of 2-deoxy-D-glucose significantly reduce the ATP content of the carotid bodies. The events postulated by the metabolic hypothesis, i.e. decrease in ATP content, release of a putative neurotransmitter and activation of the sensory nerve endings are found to occur simultaneously. A possible cause–effect relationship between these three events is discussed.

INTRODUCTION

The arterial chemoreceptors are excited by decreases in \( pO_2 \) and pH and by increases in \( pCO_2 \) and contribute to the respiratory adjustments seen in all physiological and pathological situations in which there are deviations from normality in blood gas tensions and pH. The mechanisms by which these receptors detect the changes in those blood parameters are unknown. According to one hypothesis, known as the metabolic hypothesis\(^4,15,38,39,55\) (see ref. 7 for a review), the overall transduction process in these receptors for the hypoxic stimuli will correspond to this sequence: hypoxia will decrease adenosine triphosphate (ATP) levels in the type I cells, which in turn will release a neurotransmitter capable of exciting the sensory nerve endings. This hypothesis is based on the assumption that because the metabolic poisons (inhibitors of the respiratory chain, uncouplers of oxidative phosphorylation and mitochondrial ATPase inhibitors) are potent chemostimulants they will decrease the ATP content in the chemoreceptors, as they do in other tissues. The metabolic hypothesis does not consider the mechanisms involved in the detection of the other two physiological stimuli (\( pCO_2 \), pH).

In the present study, we have tested the postulates of the metabolic hypothesis by using 2-deoxy-D-glucose (2-DG) in an in vitro preparation of the cat carotid body (CB). 2-DG is an analogue of glucose which enters the cells by the same carrier as glucose. In the presence of ATP, it is phosphorylated by hexokinase to 2-deoxy-glucose-6-phosphate (2-DG-6P) and it is not further metabolized\(^31,52\). We have chosen 2-DG to test the metabolic hypothesis because in our experimental conditions (preparations maintained in pyruvate–Tyrode free of glucose), it is an effective ATP-depleting agent, it is free of known side-actions
and its mechanism for depleting ATP (increased ATP expenditure) is different from that of the mitochondrial poisons which deplete ATP by blocking synthesis. Therefore, if 2-DG is a chemostimulant, this should allow a broader formulation of the metabolic hypothesis in the sense that a reduction in ATP levels, whatever the mechanism, is accompanied by an increase in chemoreceptor activity. We found that CBs superfused with balanced saline containing pyruvate as a nutrient instead of glucose, responded normally to different stimuli (low O₂, low pH and acetylcholine (ACh)). We found also that the presence of 2-DG in the superfusion media (Tyrode–pyruvate) caused a dose-dependent increase in the electrical activity of the carotid sinus nerve (CSN) and in the release of [³H]dopamine ([³H]DA) from the type I cells. This 2-DG-induced release was dependent on the presence of extracellular Ca²⁺. Finally, we found direct relationships amongst the chemoresponses, the amount of 2-DG phosphorylated and the decrease in the ATP content in the CB. Taken together, these findings give experimental support to the metabolic hypothesis. Some of the results have been published in abstract form²⁸.

MATERIALS AND METHODS

Surgery
Adult cats (2–3.5 kg) were anesthetized with sodium pentobarbital (30–40 mg/kg, i.p.), tracheostomized and placed on a respirator. Subsequent surgery varied slightly in the different types of experiments: (1) When the electrical activity in the CSN was to be recorded, a double ligature was placed on the external carotid artery. A section between the ligatures allowed better identification of the CSN which was dissected out and removed together with the carotid bifurcation. The block of tissue was placed in a lucite chamber filled with ice-cold 100% O₂-equilibrated Tyrode containing (in mM): NaCl, 140; KCl, 4.7; CaCl₂, 2.2; MgCl₂, 1.1; HEPES, 10; glucose, 5.5; adjusted to pH 7.4 with 1 N NaOH. (2) In the experiments in which the measurement of electrical activity was not required (experiments to measure ATP levels), a clamp was placed cephalad to the carotid sinus and another on the common carotid artery, and within 40 s (25–40 s) the bifurcation was placed in the lucite chamber as before. In both cases, the operative field was kept moist with warm saline.

Electrophysiological recording and release of [³H]dopamine
Under a dissecting scope (Leitz), the CBs with the CSN attached were prepared for recording as previously described¹⁹. In the experiments in which the release of [³H]dopamine ([³H]DA) was to be measured, the preparations were incubated for 3 h in 500 µl of 100% O₂-equilibrated Tyrode containing the catecholamine precursor [³H]-tyrosine (2.6·[³H]tyrosine; 20 Ci/mmol, Amersham) at a concentration of 20 µM and containing also 100 µM of the tyrosine hydroxylase cofactor, 6-methyl-tetrahydropterine (Sigma) and 1 mM ascorbic acid. Under these incubating conditions, the CB synthesized about 20 pmol of [³H]DA (= 4 x 10⁵ cpm) (unpublished). Whether or not the release of [³H]DA was monitored, the CBs were mounted in a drop superfusion chamber which allowed continuous flow-rate superfusion (0.4 ml/min) with different solutions at 37 °C. recording of electrical activity in the whole CSN with a suction electrode and collection of the superfusates for later analysis when needed¹⁹. The superfusates were collected in vials containing a carrier mixture (glacial acetic acid–20 mM ascorbic acid–100 µM cold DA) in the appropriate volume to yield a final pH in the superfusates of 3.2–3.6. The collection of the superfusates was made according to the following sequence: 5 min prior to the application of the stimulus (control), 5 min during the stimulus application and 4 more fractions of 5 and 10 min post-stimulus, totaling 45 min (duration of a single stimulus cycle; see Fig. 8). Normally, each experiment consisted of 4–6 stimulus cycles. The superfusates were analyzed for [³H]-labelled catecholamines released by adsorption on acid-washed alumina at pH 8.6 and elution with 1 N HCl to recover all [³H]-labelled catechol compounds⁵⁸. Positive identification of the eluted [³H]-labelled compounds was achieved by thin-layer chromatography (TLC) analysis according to Fleming and Clark²¹. Since the sum of [³H]DA and [³H]dihydroxyphenylacetic acid ([³H]DOPAC) accounted for 80–85% of the radioactivity present in the eluates (the rest being mostly [³H]tyrosine), we refer to the released material present in the eluates as [³H]DA. The stimulus-induced release was evaluated as follows: the mean re-
lease during two control periods of two consecutive cycles was taken as basal release/5 min during that cycle, and the sum of any release exceeding this basal release in all cycle fractions was considered stimulus-induced release.

The action potentials from the CSN were led through an AC-coupled preamplifier and displayed on an oscilloscope. The amplified signals were also led through a window discriminator to a digital counter-printer which read the total number of action potentials during fixed periods of time (1 or 10 s). The stimulus-induced electrical activity was evaluated similarly to the release of [3H]DA; the maximum activity elicited by a stimulus (Hz) was divided by the mean basal activity in the 5 min prior to the application of the stimulus (Hz), thus giving induced activity relative to control.

**ATP measurements**

Taking into consideration the lability of ATP levels\(^3\), special precautions were taken: (1) As mentioned before, the carotid bifurcation was removed within 40 s after clamping the common carotid artery. (2) The Tyrode in the lucite chamber was kept at 0–2 °C and renewed every 2 min to avoid warming and significant drops in O\(_2\) tension. (3) The dissection and thorough cleaning of the CB was completed within 4–6 min, but in all instances the CBs remained in the chamber for 6 min. ATP levels in both CBs of the same animal measured at this stage showed great variability, probably due to different degrees of manipulation. (4) Incubation of the CBs in 100% O\(_2\)-equilibrated Tyrode at 37 °C for 15–60 min resulted in higher ATP levels and a drastic reduction in the variability of the values obtained for both CBs of the same animal, and therefore, the CBs were systematically preincubated for 25 min at 37 °C. (5) The effect of any given stimulus on the ATP levels was assessed on one CB from one animal, the contralateral CB serving as control. Both CBs were preincubated for 25 min; the control CB was incubated for 5 additional min in the control solution and the test CB was similarly incubated for 5 min in the stimulus-containing medium. (6) The CBs were placed on pre-cooled (−20 °C) homogenizer pestles and homogenized (glass to glass) in ice-cold 0.6 N PCA (200 μl). The samples were centrifuged at 4 °C and the supernatants neutralized in the cold with 2.4 N potassium bicarbonate, and after 20 min centrifuged again. ATP was assayed in the latest supernatants either radioenzymatically or by a photoluminescence-based assay; both methods gave the same levels. \(n = 70; \bar{X} = 3.7 \times 10^{-10} \text{ mol ATP/CB}; S^2 \text{ ATP} = 2.95\). B: distribution of CB weights in the cat. Shown for comparative purposes is the distribution of CB weights from a previous study\(^2\). \(n = 104; \bar{X} = 559.0 \mu g/CB; S^2 = 47,961\). The variances of both distributions are not statistically different (\(P < 0.01\)).

**RESULTS**

**Superfusion conditions**

In preliminary experiments, it was found that in preparations superfused with 100% O\(_2\)-equilibrated Tyrode containing 5 mM glucose, only with high
2-DG concentrations (> 4 mM) in the superfusion media it was possible to obtain an increase in the CSN activity, and even this increase had a very slow onset. Superfusion with 100% O₂-equilibrated glucose-free Tyrode, on the other hand, produced a much larger and faster excitation of the CB chemoreceptors at 2-DG concentrations well below 1 mM. The use of glucose-containing Tyrode posed problems because in order to get the maximum (> 80%) excitatory effect, it was calculated on the basis of competitive inhibition kinetics that 2-DG should be raised to 20 mM. Since the CB chemoreceptors are very sensitive to osmotic changes, the findings could be misleading. On the other hand, superfusion of the CB with glucose-free Tyrode was excluded as an experimental preparation because after about 1 h (range, 20 to 80 min) in glucose-free media, there was a spontaneous increase in the CSN activity followed by unresponsiveness of the preparation to hypoxia.

As an alternative energetic substrate to glucose, pyruvate (5 mM) was tested. With pyruvate as substrate, the response to hypoxia, low pH and ACh was indistinguishable from that obtained with glucose as substrate (Fig. 2). Table 1 shows mean values of the electrical behavior of the CB when perfused with either glucose or pyruvate. Neither the basal activity nor the stimuli-induced responses were different from those obtained with glucose when the substrate was pyruvate. In addition, it was also found that the ATP levels measured after 30 min of incubation with glucose or pyruvate were not statistically different (Fig. 3). These findings, coupled to the fact that under Tyrode–pyruvate 2-DG was a powerful chemo-stimulant (see below), prompted us to use as perfusion media Tyrode–pyruvate equilibrated with 100% O₂.

**Effects of 2-DG on the chemoreceptor activity**

In Fig. 4 an experiment is presented showing the characteristics of the electrical activity obtained on

![Fig. 2. Electrical activity of the CSN of one preparation superfused with Tyrode–glucose (continuous tracings) and Tyrode–pyruvate (discontinuous tracings). The experiment started with perfusion of the CB with 100% O₂–Tyrode–glucose, and every 45 min the CB was stimulated first with low O₂ (20% O₂ balanced N₂)-containing solution, then with a 100% O₂–Tyrode–glucose at pH 6.8 and finally with a 100% O₂–Tyrode–glucose containing 5 × 10⁻⁴ M ACh. Thereafter, the superfusion was made with 100% O₂–Tyrode–pyruvate. After allowing 1 h to wash out the glucose, the same pattern of stimulation was followed. In different experiments, the order of perfusion was alternated, but in all instances the stimuli lasted 5 min (between arrows). It must be stated that the CB chemoreceptors start to increase their electrical activity when the perfusion solution is equilibrated with 60% O₂ in N₂ at the superfusion rate used in the present experiments. Under these conditions, a 20% O₂-equilibrated solution represents a hypoxic stimulus of moderate to high intensity.**

![Fig. 3. ATP levels in cat CBs incubated for 30 min in Tyrode–pyruvate (striped bar) or in Tyrode–glucose (clear bar). Pyruvate: n = 14; X = 3.76; S.E.M. = 0.37. Glucose: n = 19; X = 3.48; S.E.M. = 0.37. All CBs of this figure were assayed by the luciferin–luciferase method. Comparisons: t-test, P > 0.05.](image-url)
TABLE I
Characteristics of the CB response to different stimuli during superfusion with glucose (G) and with pyruvate (P)

In all cases, the window discriminator was adjusted at the onset of the experiments to provide a basal activity (superfusion with 100% O2-equilibrated solution) in the range of 25–50 action potentials. The counting windows remained unchanged for the rest of the experiment. Data represent means ± S.E.M. No statistical differences (t-test) were observed in any of the parameters evaluated.

<table>
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<td>P (n = 7)</td>
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<td>38 ± 5</td>
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superfusing the CBs for 5 min with different concentrations of 2-DG. Note (Fig. 4 inset) the dose-dependent response. Note also that the onset and the time to peak of the response decreased with increasing concentrations of 2-DG. And finally it can be seen that the recovery time (time for the electrical activity to return to the pre-stimulus levels) increased with increasing 2-DG (Fig. 4).

Fig. 5 shows dose–response curves for the effect of 2-DG on the electrical activity of the CSN. The upper curve was obtained from 10 CBs which immediately upon dissection were mounted in the superfusion-recording chamber. The lower curve was obtained from 8 CBs which had been incubated in Tyrode-glucose for the 3 h period prior to electrophysiologic recording (Fig. 5). Since this difference in the response upon incubation is not observed with other stimuli (hypoxia, low pH, CN⁻), it appeared that it must have been related to some specific factor concerned with the mechanism of action of 2-DG. In this regard it must be mentioned that in two structures (the superior cervical ganglion and the adrenal medulla) embryologically similar to the type I cells, there is an increase in the stores of glycogen upon incubation of the structures in vitro with 5 mM glu-
It may be that the breakdown of the glycogen during the recording period generates glucose, which competes with 2-DG at the phosphorylation step (see Discussion; see also Fig. 9).

Effects of 2-DG on the release of [3H]DA from the type 1 cells: correlation with the electrical activity and ATP levels

It is well-documented that the exocytotic release of neurotransmitters and hormones is a process which requires ATP. It is also well-documented that in a great variety of secretory structures (brain tissue, adrenal gland, sympathetic endings, platelets), a decrease in ATP levels reduces rather than increases the secretory process. Since the above facts are opposite to what the metabolic hypothesis predicts for the chemoreceptors, it was of great interest to establish first if 2-DG induced the release of [3H]DA from type 1 cells, and second if 2-DG reduced the ATP levels in the CB.

Fig. 6A shows a single experiment in which it is evident that 2-DG applied for 5 min induced a release of [3H]DA from a preloaded CB which was dependent on the concentration of the glucose analogue. Fig. 6B shows the dose–response curves for both the release of [3H]DA and the electrical activity obtained from 8 experiments.

Fig. 7 shows the effects of 2-DG (1, 2 and 4 mM) on the ATP levels in CBs, compared to contralateral organs similarly incubated in the absence of 2-DG. Fig. 7 also shows the relative rates of 2-[3H]DG phosphorylation in the range of concentrations from 0.25 to 4 mM. The data presented in these last two figures (Figs. 6 and 7) certainly suggest that in the CB chemoreceptors there is a close relationship between the decrease in the ATP levels, the release of a putative neurotransmitter and the genesis of activity in the CSN, as the metabolic hypothesis proposes.
Characteristics of the 2-DG-induced release of [3H]DA

Time-course of [3H]DA release. As shown in Fig. 4, the electrical activity elicited by 2-DG outlasts its application, probably because the phosphorylation of the accumulated 2-DG continued within the cells. Therefore, it was of interest to correlate the time-course of 2-DG-elicited electrical activity in the CSN with the time-course of the 2-DG-induced release of [3H]DA. In Fig. 8 is shown the time-course of the 2-DG-induced release of [3H]DA. Note that the fraction with highest [3H]DA is the post-stimulus period, despite the fact that with 2 and 4 mM (see inset in Fig. 4), the peak of electrical activity occurred during the stimulus period. This apparent delay in [3H]DA release should in fact be considered the result of washout of released [3H]DA. Note also in Fig. 8, that the time required for [3H]DA release to return to pre-stimulus levels is about the same as that required for the electrical activity to recover to the basal level (compare Figs. 4 and 8). All the parallelism between 2-DG-elicited electrical activity and [3H]DA release suggest that DA plays a prominent role in the genesis of the chemosensory activity, as already pointed out by other investigators. 

Ca²⁺ dependence of the 2-DG-evoked release of [3H]DA. The Ca²⁺ dependence of the 2-DG-evoked release of [3H]DA was studied in 4 experiments, and the results are presented in Fig. 9. In these experiments, the first and fourth cycles were made while perfusing the preparation with normal Ca²⁺-Tyrode, and the second and third cycles with 0 Ca²⁺-Tyrode. Note that the release of [3H]DA in the two control cycles (Fig. 9A; Nos. 1 and 4) is quite different, being much more pronounced in the last cycle. As already mentioned (see Fig. 5), this difference probably is related to the slow depletion of the store of glycogen that was built up during the period of incubation prior to the recording period. Note also that, on the average, the 2-DG-evoked release of [3H]DA in the two cycles with 0 Ca²⁺ is 141% of the basal release in these same cycles, while in the two control cycles the evoked release is 320% of the basal release (P < 0.01). When 1 mM EGTA is added to the 0 Ca²⁺ media, there is no evoked release (not shown). The average electrical activity induced by 2-DG was also reduced in the two cycles with 0 Ca²⁺ (Fig. 9B). Thus, it represented 175% of the basal activity in 0 Ca²⁺ and 273% in the control cycles (P < 0.02). In the presence of EGTA, the electrical activity is un-
DISCUSSION

The present work has investigated the metabolic hypothesis of chemoreception by correlating the effects of 2-DG on the ATP levels in the CB with the release of a putative neurotransmitter (DA) by the type I cells and with the electrical activity in the CSN. As the metabolic hypothesis postulates, there is a direct and close relationship between the decrease in ATP and the increase in DA release and CSN activity.

**ATP levels in the CB**

The mean ATP content found in our sample \( n = 70 \) of CBs is \( 3.7 \times 10^{-10} \text{ mol/CB} \), with a distribution which was not statistically different from that of the CB weights. The estimated intracellular concentration of ATP in the CB is about 2–3 mM, a concentration quite similar to that found in brain tissue and liver. Our findings contrast with the data recently reported by Acker and Starlinger. These authors reported ATP levels of \( 0.087 \pm 0.056 \) (S.D.) \text{ nmol/CB} \( (n = 31) \), that is, values only about 24% of ours. In the same article, it was also reported that severe hypoxia \( (\text{p}O_2 = 25 \text{ torr during 11 min}) \) did not modify the ATP content in the CB. Again, this observation contrasts with our previous findings, showing that a moderate to intense hypoxic episode (incubation in air-equilibrated Tyrode for 5 min) significantly reduced the ATP content in the CB. It is difficult to reconcile both sets of data since they were obtained from the same animal species; however, as pointed out in Materials and Methods (see also ref. 35), the ATP stores are very labile, and any mechanical and/or hy-
poxic trauma to the CB tissues can lead to rapid depletion of metabolically active ATP deposits. Since Acker and Starlinger assayed ATP in the CB directly upon removal from the animals, and considering the small size of the organ, it may be that their dissection maneuvers were responsible for the lower and greatly dispersed values which they reported. If this were the case, it is conceivable that they would not have observed a further ATP depletion following a hypoxic episode.

The decrease of the ATP levels observed in CBs incubated with pyruvate in the presence of 2-DG was expected considering that 2-DG is a good substrate for hexokinase and that 2-DG-6P is not a substrate for glucose phosphate isomerase. The decrease in ATP observed in the whole CB under 2-DG should result from a reduction in ATP in all glomic structures probably in direct proportion to their metabolic rate. Because the type I cells are considered the CB structures with the higher metabolic rate, they probably contribute significantly to the observed overall ATP reduction. Nonetheless, enough ATP must remain within these cells to support the exocytotic Ca\(^{2+}\)-dependent release.

**2-DG as a chemostimulant**

Any agent (metabolic poison) which interferes with the generation of phosphate-bond energy is a stimulant of the CB chemoreceptors. Although the metabolic poisons have many side effects, seldom considered in the context of the chemoreception process, the fact that all of them are known to decrease the ATP/ADP quotient in other structures and are powerful stimulants of the CB chemoreceptors has been the basis for the proposal of the metabolic hypothesis (see refs. 7 and 17 for reviews). As was stated in the introduction, 2-DG was chosen for this study because of the apparent lack of side-effects and because it will lead to ATP depletion by increasing ATP expenditures rather than by blocking ATP synthesis, especially under our conditions of superfusion with Tyrode-pyruvate. The differences in the dose–response curves of 2-DG on the CSN activity in non-incubated or incubated CBs are, as already mentioned, in all probability due to the accumulation of glycogen during the incubation period. In addition to the observations made in the superior cervical ganglion and adrenomedullary cells, which show that on incubation in vitro with glucose there is an increase in glycogen deposits, we have shown that in CBs superfused with glucose-free Tyrode, the time required to abolish the hypoxic response is much longer if the organs are previously incubated for 3 h in Tyrode containing 5 mM glucose. This delay can only be interpreted as the result of a buildup of energetic stores during the incubation period. In keeping with this notion is also the fact that the 2-DG phosphorylation curve, which has a hyperbolic shape (the correlation coefficient of 1/V (rate of phosphorylation) vs 1/S (2-DG concentration) is 0.998), has an apparent K\(_m\) of 2.2 mM, which is much higher than that of hexokinase for 2-DG, which suggests a competitive inhibition of glucose with 2-DG even in these CBs that had not been previously incubated.

**Correlation between ATP levels, release of \[^{3}H\]DA and CSN activity**

The data presented in Fig. 6 and in subsequent figures show a close correlation between ATP levels, release of \[^{3}H\]DA and CSN activity. The metabolic hypothesis postulates such a correlation, and even more, postulates a cause–effect relationship between the decrease in the ATP levels, the release of the neurotransmitter and the genesis of activity in the CSN. A cause–effect relationship between the decrease in the ATP levels and the release of the putative neurotransmitter DA seems to exist because hypoxia and CN\(^{-}\), both of which decrease the ATP content in the CB, also induce a release of DA which is Ca\(^{2+}\)-dependent. How a decrease in the ATP levels triggers the entry of Ca\(^{2+}\) into the type I cells is not known, but depolarization of the type I cells seems to be involved because organic and inorganic blockers of the voltage-dependent Ca\(^{2+}\) channels inhibit the hypoxic as well as the high K\(^{+}\)-induced release of DA. These observations raise another question: how does the decrease in the ATP levels induce depolarization of the type I cells. In this regard, it is necessary to propose that an ATP-dependent electrogentic mechanism contributes to the maintenance of the membrane potential, as is also the case in other structures (see refs. 9 and 20). Consistent with this suggestion are the following observations: (1) Ouabain releases \[^{3}H\]DA from the type I cells, an effect which is fast in onset and occurs at lower ouabain concen-
tration than in the sympathetic endings.  

2) Ouabain depolarizes the type I cells.  

3) Cooling of the CBs also induces depolarization of the type I cells, and ouabain partially prevents the depolarization induced by lowered temperatures.  

Taken together, these observations can be interpreted as due to the presence in the type I cells of an ouabain-sensitive hyperpolarizing Na\(^+\)-K\(^+\) pump, which on reduction of the ATP/ADP quotient loses its electronegativity, leading to depolarization.  

The depression of such a pump by cooling or its inhibition by ouabain will lead to depolarization.

The possible relationship between the release of putative neurotransmitters and the genesis of activity in the CSN remains to be considered. In all likelihood, all of the neuroregulators present in the type I cells (including, in addition to DA, ACh, norepinephrine, Leu- and Met-enkephalins, substance P; see ref. 17) are coreleased with DA.  

The precise role of these neuroregulators in the chemoreception process is far from clear (see refs. 17 and 36). It is well known that exogenously applied DA can produce excitation or inhibition of the ongoing CSN activity, depending on the dose and on the animal species, but as stated by McQueen, the real problem lies in establishing whether exogenously applied DA has similar actions to that which is endogenously released within the tissues. On the basis of pharmacological experiments, Docherty and Nolan et al. have suggested that endogenous DA may in fact be excitatory, contrary to their previous suggestions. The findings reported in this and in previous works, correlating synthesis and release of \(^{[3]}\)HDA with the activity in the CSN fit with the notion that DA plays a role in the genesis of the CSN activity, as suggested by Leitner et al.

In conclusion, as the metabolic hypothesis postulates, our results show that there is a linkage between ATP decrease in the CB and increase in CSN activity, which appears to represent the physiological mechanism for chemoreception of hypoxia. In all likelihood, this ATP-coupled mechanism is not the only one present in the CB. Alternative pathways currently under investigation appear to be responsible for chemoreception to low pH and high pCO\(_2\).

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