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MECHANICAL SENSITIVITY OF CAROTID BODY GLOMUS CELLS

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Abstract

Cultured glomus cells from rat carotid bodes were prepared for optical studies of intracellular calcium using the Fura-2 dye. The baseline calcium had a mean of about 40 nM showing either a relatively steady level or large calcium spikes. Some cells did not show measurable levels of $[Ca^{2+}]_i$. Stirring the fluid bathing the cultures induced large increases in $[Ca^{2+}]_i$ which were abolished when the bathing medium had zero Ca^{2+} and EGTA. It is concluded that glomus cells respond to mechanical stimulation when directly exposed to this stimulus and are not protected by supporting structures. It is unknown if the electrical properties of these cells are also affected by mechanical challenges.

Keywords

Carotid body; glomus cells; calcium; mechanical stimulation

1. Introduction

Carotid body chemoreceptors are mainly responsive to chemical changes in their environment, while carotid sinus baroreceptors respond specifically to mechanical deformation of their nerve endings. However, this distinction is not absolute as detailed in the DISCUSSION. Eyzaguirre and Zapata (1968) found that stirring the fluid bathing an *in vitro* carotid body markedly changed the discharge frequency of chemoreceptor afferents. When the baseline discharge was low during high oxygenation, stirring increased it. During flow interruption, when the discharge was high, stirring induced discharge depression. This effect was interpreted as being the result of disturbing the chemical environment surrounding the receptors. There is ample evidence that neurochemical agents are released from *in vitro* carotid bodies at rest and during stimulation (Eyzaguirre and Zapata, 1968). This has led to numerous studies in search of neurochemical mechanisms of chemoreception (see Gonzalez et al, 1994).

The purpose of this study was to see if stirring the fluid bathing cultured glomus cells could affect their behavior. For this purpose we chose preparations used for intracellular calcium studies. We found that disturbances of the medium induced large increases in intracellular

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calcium in most, not all, glomus cells. Functional implications and possible mechanisms of action are presented in the DISCUSSION.

2. Methods

The methods used in this study for calcium measurements were identical to those previously published (Abudara et al., 2001; Jiang and Eyzaguirre, 2004). Briefly, carotid bodies were removed from anesthetized rats and rinsed in sterile Hank's solution. The tissues were then immersed in an ice-cold physiological solution for cleaning and dissection. The tissues were then placed in Ca^{2+} and Mg^{2+} -free Hanks' medium at room temperature for 30 min before moving them to Hank's solution containing 0.3% Type II collagenase for 40 min. at 37° C. After washing in Ham's F12, the carotid bodies were gently dissected in this medium, which also contained 80 U/l insulin 10% bovine serum, 1% antibiotic-antimycotic at pH 7.43. The cells were then plated onto poly-L-lysine coated glass cover slips and kept in an incubator at 37°C for 1-2 h for adhesion to the glass surface.

The cover slips containing the cells were mounted in a 100 µl chamber for superfusion (0.5-1 ml/min) with physiological saline flowing through a 500 µl loop, exposing the tissues for 20-30 sec to different environments.

For $[\text{Ca}^{2+}]_i$ ratiometry, the tissues were loaded with 1 µM of cell permeant Fura-2 AM in 0.12% of the nonionic surfactant pluronic F-127 for 10-20 min. at 37° C, and the preparation was mounted on the stage of an inverted digital fluorescence microscope (Attofluor Ratio Vision system, Zeiss) equipped with a CCD camera and computer. Cells were viewed with a 40x immersion objective and light excited through intermittent 334 and 380 nm filters used in conjunction with a single 520 nm emission filter. The system permitted selecting single or clustered (up to 99) cells for photography and analyses. Images and ratios were displayed through an Attograph-Attoview for a Windows program. Calcium values were recorded every few seconds up to several minutes.

Experimental fluorescence ratios (334/380) were converted to $[\text{Ca}^{2+}]_i$ after calibration, using the formula:

$$[\text{Ca}^{2+}]_i = K_d Q [R - R_{\min}] / [(R_{\max} - R)]$$

where K_d is the Ca^{2+} dissociation constant of the dye; Q is the fluorescence ratio (min/max) at 380 nm; R is the experimentally obtained fluorescence intensity ratio; $R_{\min}$ is the fluorescence ratio given by the lowest calcium solutions; $R_{\max}$ is the ratio obtained with the highest calcium concentration.

3. Results

The calcium content at rest showed three different patterns. One was a steady baseline with a median of about 50 nM ranging from 7 nM (10th percentile) to 83 nM (90th percentile), as shown in Fig. 1A. Another was an unstable baseline in which large calcium spikes appeared with some regularity, as in Fig. 1C. In other cases, no calcium was detected in the glomus cells (Fig. 1D). This may be due, at least in some cases, to a level of Fura 2 inside cells below the level of detection of the system employed, since some of these cells responded to stirring with a Ca^{2+} signal.

Stirring the fluid covering the cultures was done by gentle sucking and ejecting fluid through the fine tip of a suction pipette. This procedure induced a large increase in intracellular calcium

as shown in Fig. 1A, where each stirring is represented by a solid arrow. It should be noted that the first stirring produced the largest effect, from a baseline of about 80 nM to a peak of about 900 nM. Subsequent stimuli were considerably less effective. This decline in effectiveness was quite common and appeared to be caused by some sort of receptor fatigue.

Mechanical stimulation was also performed by touching cells with the tip of the pipette, advanced through the micro manipulator, and by creating turbulence in perfusion flow by opening and closing the one-way valve at the entrance of the perfusion system. These procedures evoked similar Ca^{2+} responses to those described above.

An immediate concern was to see if the stirring effects were an artifact produced by disturbing the light path in the system. We thought that it might not be the case since some cells were unaffected by fluid stirring. But, to be certain we bathed the preparations in zero calcium + 1 mM EGTA. This is shown in Fig. 1B. The calcium baseline was not totally stable since some small calcium spikes were seen. The first stirring (solid arrow) induced a burst of calcium spikes that disappeared and the calcium baseline reached zero, when calcium was removed from the medium (dotted horizontal bar). Subsequent stimuli (open arrows) were ineffective, showing that disturbing the light path in our system did not produce undesirable artifacts.

When the calcium baseline was unstable and showing large calcium spikes, stirring was less effective as shown in Fig. 1C. The first stimulus was ineffective (open arrow) and the preparation showed a series of spontaneous and large calcium spikes. The next three stirrings (solid arrows) induced calcium spikes, which were much smaller than the spontaneous ones. Afterwards, the preparation became relatively quiet and two further stirrings (open arrows) were ineffective.

When no calcium was detected in some glomus cells (Fig. 1D) stirring of the medium was often capable of inducing calcium influx.

An interesting characteristic of the previously described stirring effects was that influxes of calcium seemed to occur at the same time in different cells of a given preparation as illustrated in Fig. 2. The stacked areas show the intracellular calcium baseline of four glomus cells. The first stirring increased $[\text{Ca}^{2+}]_i$ of three cells while the second one increased it in all cells, with larger effects on the previously affected ones. The most important point of this experiment is to show that intracellular calcium increases occurred at the same time in all cells. This happened in this and all other experiments. trials the results obtained in 16 experiments, compiling observations from 400 cells, the calcium baseline was 40.3 ± 7.8 nM (mean $\pm$ SE), $[\text{Ca}^{2+}]_i$ increased in response to stirring by 457.6 ± 111.4 nM, and durations of increases in intracellular calcium (open time) were 23.3 ± 4.7 sec.

4. Discussion

When recording from the carotid sinus nerve, a common practice to distinguish chemoreceptor afferents from baroreceptor fibers is to tap or press on the carotid bifurcation area. Baroreceptors increase their discharge whereas chemoreceptors do not. However, chemoreceptors are not entirely unresponsive to mechanical stimulation. *In vitro*, when the bathing medium is made hyperosmotic, glomus cells depolarize and there is an increase in chemoreceptor discharges. The opposite happens when the medium is made hyposmotic (Gallego et al., 1979). *In situ* preparations behave differently, since the chemoreceptor discharge decreases when the blood becomes hyperosmotic (Gallego and Belmonte, 1974). These authors suggested that the chemoreceptor response may have been distorted by accompanying vascular effects. Despite differences *in vivo* and *in vitro*, it is clear that chemoreceptors respond to some form of mechanical deformation that eventually will affect the generation of nerve impulses.

It must be mentioned that, working on cat carotid bodies superfused *in vitro*, Alcayaga et al. (1988) observed that changes in superfusion flow determined inverse changes in the frequency of chemosensory discharges recorded from the carotid sinus nerve, when all other natural chemoreceptor stimuli were held constant.

The above experiments suggest that when the supporting structures (connective tissue, capsule, etc.) of the carotid body are intact, external mechanical stimuli are ineffective. In this situation, only mechanical challenges from within the organ (in or around the blood vessels) can affect the nerve discharge.

The present study shows that exposed glomus cells are sensitive to mechanical disturbances of their medium. Since only calcium fluxes were measured, we still do not know whether other cell parameters, such as electric responses, and nerve ending are also affected by mechanical stimuli.

Interestingly, simultaneous increases in Ca^{2+} occurred in neighbor cells, as reported in Results. One wonders if such simultaneous effects are mediated by intercellular coupling, which has been amply demonstrated in the carotid body (for references see Eyzaguirre, 2007).

At this point, we do not know what type of membrane channels are responsible for calcium influx induced by mechanical disturbance of the medium surrounding cultured glomus cells. We know that the phenomenon is relatively brief and bi-directional since there is no accumulation of calcium when glomus cells are mechanically disturbed. Molnar et al. (2003) have suggested that hyposmotic challenges increase $[\text{Ca}^{2+}]_i$ by depolarizing glomus cells, activating voltage-gated calcium channels. However, cat glomus cells are hyperpolarized in hyposmotic solutions (Gallego et al., 1979).

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Figure 1.

A-D, mean effects of stirring the fluid bathing cultured glomus cells on calcium influx. Solid arrows, stirring induces calcium influx. Open arrows, ineffective stirrings. A, effects on stable calcium baseline. Note reduction of effect after three stimuli. B, burst of calcium influxes after one stirring. Effect disappears in zero calcium + 1 mM EGTA. C, preparation showing spontaneous calcium spikes. Stirrings induce calcium influxes no larger than the spontaneous activity. D, calcium influx produced by stirring in preparation showing no baseline calcium. N, number of recorded cells.

Figure 2. Superimposed areas depicting calcium fluxes in four cells. Two stirrings (bottom line) separated by about 50 s induce simultaneous effects on calcium influx. Note that calcium influx occurs simultaneously in all activated cells, as in all experiments.