Calcium Sensitivity for Hypoxia in PGNs with PC-12 Cells in Co-Culture

G.P. Patel, S.M. Baby, A. Roy and S. Lahiri

Abstract Calcium sensitivity of petrosal ganglion neurons (PGNs) to chemical stimuli with and without PC-12 cells in co-culture instead of glomus is not known – the idea being that two types of unusual cells could form synapse and provide a model for studies of chemotransduction. Calcium levels in the PGNs were measured in the presence of different chemical stimuli in the bath medium. Remarkably, the PGNs alone were not sensitive to hypoxia (10 torr), PCO (~300 torr in normoxa) nor to ATP (100 μM) but they developed the sensitivity to these stimuli in synaptic contact with PC-12 cells. The sharp rise in calcium level was suppressed (2/3) by suramin (100 μM), a purinergic blocker, and the remaining 1/3 was blocked by hexomethonium, a cholinergic blocker. Taken together, these observations suggest that PGNs developed neurotransmission when in contact with PC-12 cells, as if the latter substituting for glomus cells, thus providing a model for chemotransduction studies. The reason for the insensitivity of PGNs alone to the chemical stimuli is unknown at this time.

Keywords Co-culture of PGNs and PC-12 cells · Hypoxia · High PCO · PGNs · PC-12 cells · Calcium sensitivity

1 Introduction

Alcayaga et al. (2003) showed that natural stimuli of the carotid body (CB) cell have no effect on PG neurons (PGNs), but the effects on PGNs can be recorded only in co-culture with carotid body cells. The synaptic contacts between CB cells and PGNs appear to be necessary for the generation of chemosensory activity. However,

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no absolute correlation between the sensory activity and the electrical properties of the neurons has been found (Zhong et al., 1997; Zhang et al., 2000). Thus, there remains an uncertainty as to whether the PGNs respond with or without glomus cells connection.

We tested the possibility using another preparation, i.e. co-culturing PGNs with PC-12 cells, because PC-12 cells have been shown to have properties similar to glomus cells (Seta and Millhorn, 2004). We found that PGNs developed sensitivity to hypoxia only in presence of PC-12 cells, arguing that synaptic contact between PC-12 cells and PGNs was an absolute necessity.

In the co-culture of rats PGNs and CB cells, basal discharge and hypoxia-induced action potential are partially blocked by suramin (purinargic blocker) or hexomethonium (cholinergic blocker) but completely blocked by simultaneous application of both blockers (Zhang et al., 2000). Thus functional synapse and PGNs and glomus cells is a real possibility. Like glomus cell, PC-12 cells are also oxygen sensitive and are excitable. Both originate from neural crest and stimulated by hypoxia which is translated into an increase in intercellular calcium and neurotransmitter release.

Several molecules including acetylcholine, ATP, dopamine etc. have been proposed to participate in the synaptic transmission (Gonzalez et al., 1994; Iturriaga and Alcayaga, 2004; Lahiri et al., 2006; Nurse, 2005). To investigate the synaptic mechanism we have developed and tested a co-culture model of PC-12 cells and PGNs for chemical synapse. Results of this investigation have been presented in this paper.

2 Methods

PGNs were co-cultured with or without PC-12 cells. Intercellular calcium of PGNs was measured with Fura-2 (Roy et al., 2004). The idea being that calcium was increased in the PGNs when in contact with PC-12 cells only.

3 Results and Discussion

The summarized results are presented in the following table (Table 1). The scenario that the PGNs, which were in contact with the PC-12 cells, responded to hypoxia, high PCO, and the application of ATP (Figs. 1 and 2); whereas the PGNs which were cultured without PC-12 cells remained quiescent to the same stimuli (Fig. 2). This results point to the importance of PC-12 cells in the co-culture and are consistence with those of Alcayaga et al. (2003).

A co-culture between PGNs and glomus cells has been used before with hypoxia with similar results (Nurse, 2005).

The reason for the lack of response of the PGNs is not obvious. It is hypothesized that the receptors for the response were absent in PGNs.

This effect of CO resembles those of hypoxia.

Table 1 Summary responses in isolated PGN and co-cultures of PGN +PC12 cells

Stimuli	Effects	Effects
	[Ca2+]i	[Ca2+]i
Hypoxia	↑	No response
ATP	↑	No response
CO	↑	No response
CO + Suramin	↓	No response
CO + Suramin + Hexamethonium	↓	No response

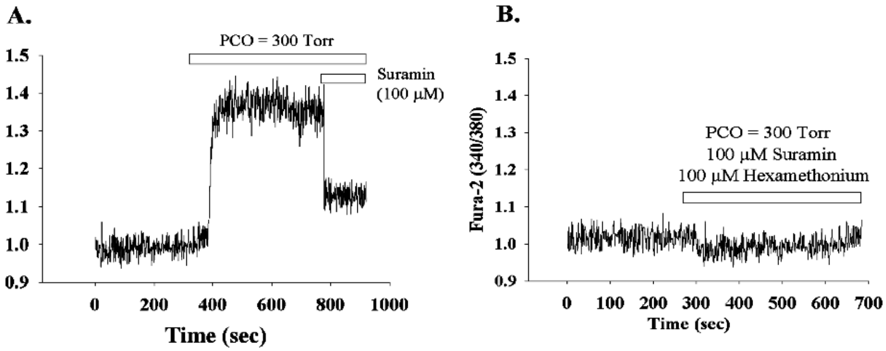

Fig. 1 Example of the effect of CO (Pco = 300 Torr) on intracellular [Ca^{2+}] response of PGN co-cultured with PC-12 cells, **A**. Increase in intracellular Ca^{2+} of PGN co-cultured with PC-12 cells during CO is partially inhibited (75%) by suramin (100 μM). **B**. Combined application of suramin and hexamethonium completely inhibited the intracellular [Ca^{2+}] response of PGN

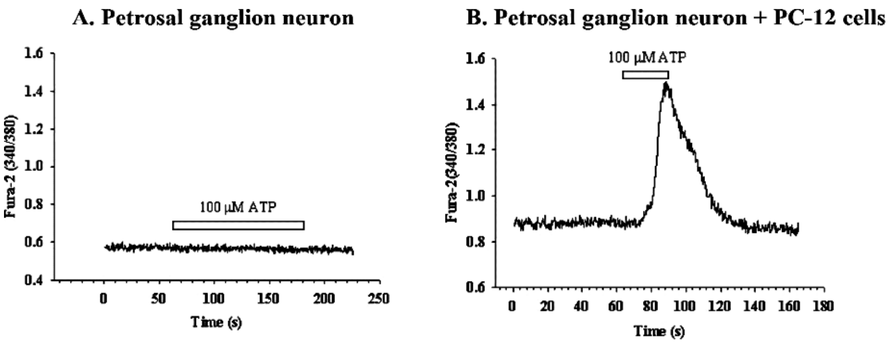

Fig. 2 Effect of ATP on intracellular [Ca^{2+}] of PGNs cultured without (A) and with (B) PC-12 cells. Increase in intracellular Ca^{2+} of PGN co-cultured with PC-12 cells with ATP suggests transduction of the stimulus through functional synapse (**B**). However, PGNs without PC-12 connection remain quiescent to ATP stimulus (**A**). This suggests that ATP, acting via postsynaptic purinoceptors, excites the PGN

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