Vascular smooth muscle sensitivity to varying oxygen tensions, Bay K 8644 and Nifedipine

***A.C. Ugwu and J. C. McGrant**

**Smooth Muscle Physiology Unit, Department of Physiology, School of Medicine, College of Medical Sciences, University of Benin, Benin City, Nigeria.*

[1]Institute of Biomedical Sciences, University of Glasgow, Glasgow G12 8QQ. U.K.

Summary

With the suggestion that while high oxygen levels may alter the response beyond the receptor, with wrong information about excitation-contraction coupling; it was observed that levels of oxygen affect both the calcium channel blockers and facilitators with net reduction of influx or utilisation of external calcium. In this our study, using rat tail artery activated by noradrenaline and potassium chloride in the presence of Bay K. 8644 and nifedipine at different oxygen levels, we showed that desensitisation of the responses of the vascular smooth muscle occurred. It was evident that the underlying basis of vascular responses observed with different oxygen levels was apparently due to shifting of the control curves. Hence, drugs which modify calcium channels functions may in vitro, be altered qualitatively and quantitatively by hyperoxic conditions, in receptor or voltage sensitive calcium channels, since oxygen appears to exert facilitatory effects on calcium channels.

Résumé

D'après la suggestion ayant rapport au niveau élevé d'oxygène que peut changer la réponse au délà du récepteur, avec des informations fausses sur l'accouplement-contraction d'excitation; on a remarqué que les niveaux d'oxygène avaient l'influence sur le calcium et les facilitateurs tous les deux, avec la nette réduction ou l'utilisation du calcium externe.

Dans notre étude, tout en utilisant l'artère de la queue du rat activé par noradrenaline et chlorure depotassium avec le Bay K 8644 et nifedipine dans les niveaux diverses d'oxegène, nous avons pu demontrer que la désensibilisation de la réponse du muscle doux vasculaire s'est produite. Il était evident que le principle fondamental des réponses vasculaires remarquées avec les niveaux diverses d'oxegène étaient apparemment causés par le changement de la limitation de la curbe. Alors, les drogues qui modifient les fonctions de la filière du calcium peuvent, en vitro, en vitro, être changé qualitativement et quantitativement par des conditions hyperoxiques dans le recépteur ou la tension sensible de la filiére du calcium puisqu'il parait que l'oxygéne excerce une pression facilitoire sur les filières du calcium .

Introduction

The modulatory effects of different levels of oxygen in altering the responses of smooth muscles to different activating agents have been extensively studied by various groups of workers and they agree that reduction of the PO_2 (oxygen partial pressure) in the fluid bathing smooth muscle preparations may have some effect on their responsiveness to drug or to nerve stimulation in different organs[1,3,5,7,8,9].

It is possible that high oxygen tension alters the response beyond the receptor and this may provide false information on excitation - contraction coupling. It is suggested that noradrenaline susceptibility to nifedipine, which is a calcium entry blocker, was reduced by low oxygen partial pressure[4]. Similarly, it has been suggested that oxygen tension may reduce net influx or utilization of external calcium in vascular smooth muscle[2].

The aim of this study was to examine desensitisation and the variations of oxygen tension, Bay K 8644 and nifedipine in a longitudinal study of a vascular smooth muscle activated by noradrenaline (3μM) and by KCl (100mM).

Method

Lengths of the proximal segment (1–2cm) of tail artery (male Wistar rat, 300–350g) were perfused, at 2-3ml/min with Kreb's bicarbonate with (Ca^{2+}) 2.5mM; PO_2 95% O_2(control); 16% O_2 and 4% O_2; pH 7.2–7.3 at 37°C), using a pulsatile flow pump and was immersed in a similar medium.

Maximal changes in the peaks of the pulsatile perfusion pressure waves were measured for calculation of vasoconstrictor responses.

Concentration – response curves (CRCs) for Ca^{2+} (otherwise referred to as CCRCs) were constructed by activating the tissue with noradrenaline (NA) (3μM) or potassium chloride (KCl) 100mM with or without Bay K 8644 (0.1μM) or nifedipine (0.1μM).

NA or KCl was added when $[Ca^{2+}]$ was low (1μM) and $[Ca^{2+}]$ was increased in steps allowing construction of CCRCs as an estimate of $[Ca^{2+}]_0$ sensitivity.

$[Ca^{2+}]_0$ was buffered with nitrilotriacetic acid (NTA) and EGTA (2.5mM each), so that the "total Ca^{2+} = 2.5mM to 10mM but $[Ca^{2+}]o$ = 1μM to 5mM. The buffers allow accurate determination of $[Ca^{2+}]o$ (otherwise impossible when less than 0.1mM; (McGrath et al., 1984).

Each curve took about 45 min. to construct at a given oxygen tension and it was separated from the next curve by 15 min. equilibration period in between curves. The gas bubbling was carried out in sequence starting with 95% O_2 then 16% O_2 and finally 4% O_2 in the SAME set of tissues - hence the longitudinal study. Bay K 8644 (0.1μM) and nifedipine (0.1μM) were then tested in the same sequence.

Shifts of the CCRCs at the level of 30% of the first control maximum response (EC_{30s}) were plotted as - Log EC_{30} against time (*min*).

Thus this EC_{30}, as we called it, is steadily increasing (or moving to the right of the graph) with subsequent curves (Figure 1a). This value was further expressed as the negative logarithm, so that the rightward shift will represent a falling index of sensitivity (Fig. 1b). Our graphs on the sensitivity of the tissue to calcium are thus drawn to show the - log EC_{30} values against time in minutes in order to see what happens to consecutive curves in a series (Fig. 1b). With this approach, it was easy to follow the rate of desensitisation in the vascular smooth muscle.

The results were expressed, or represented on graphs, as the mean ± SEM. Statistical analysis was performed using student's test and the 0.05 level of probability was regarded as significant.

*Correspondence

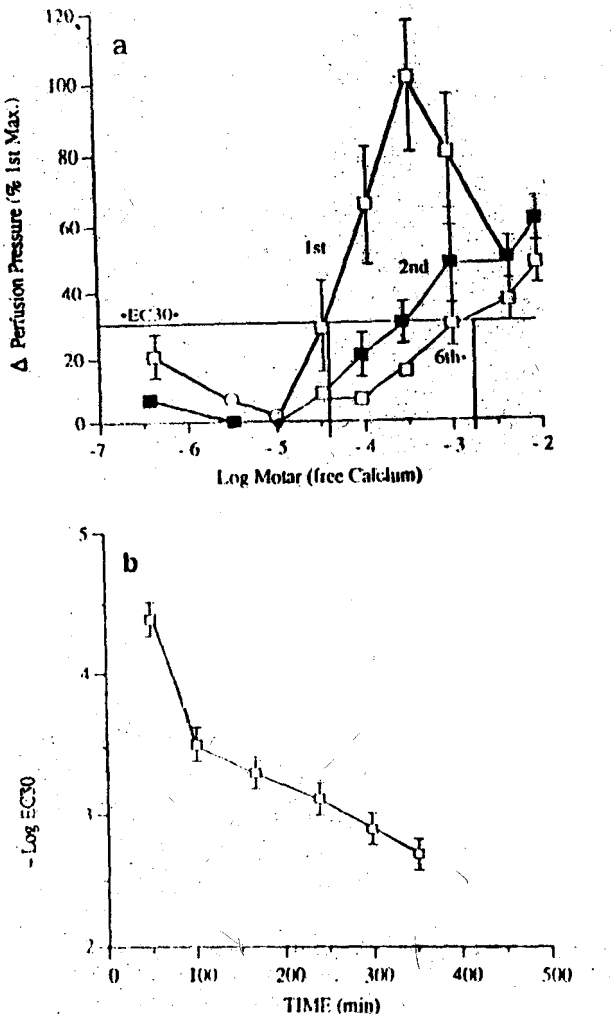

Fig. 1 ***Illustration of the response level (EC_{30}) shown for assessment of Ca^{2+} sensitivity. The EC_{30} shifted to the right with subsequent curves (a) and this represented as a steady decline in calcium sensitivity when - log EC_{30} values plotted against time in mins (b).***

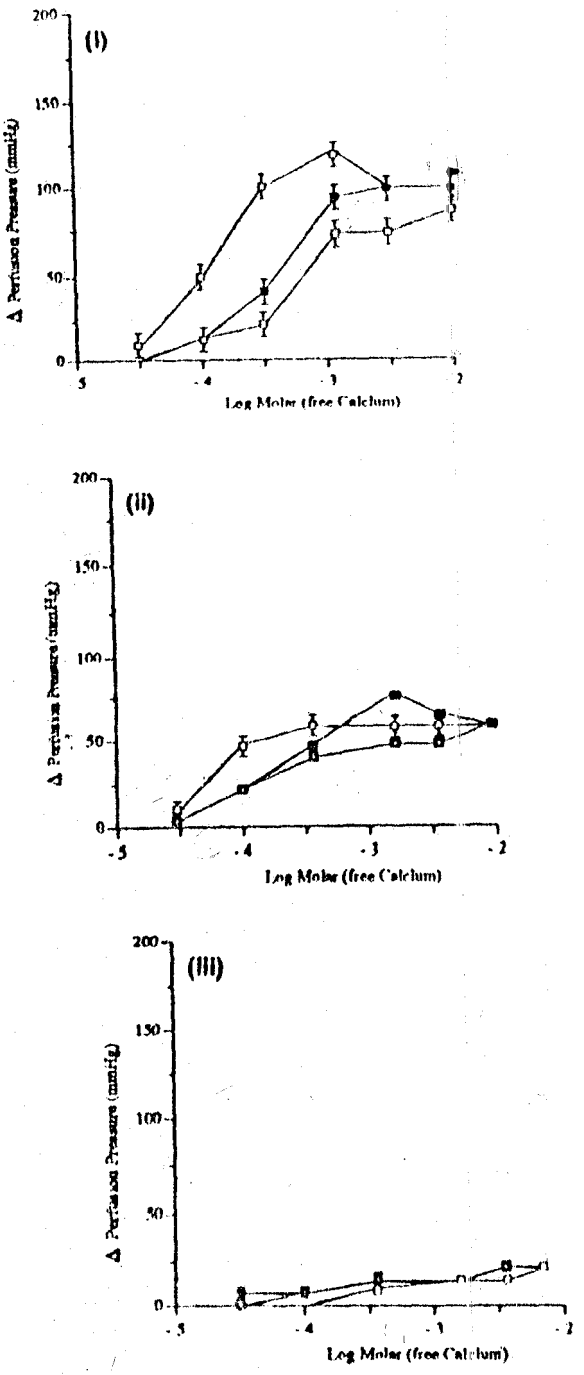

Fig. 2 ***Sequential CCRCs constructed in three oxygen gas tension of 95%, 16% and 4% (i) represents control curves; (ii) is in the presence of Bay K8644 and (iii) is in the presence of nifedipine in NA-induced responses of the rat tail artery.***

Results

When the oxygen tension was varied sequentially from 95% to 16% and then to 4% for the time control curves, it was observed that the plot of – log EC_{30} values against time (min) clearly shows that the second and subsequent CCRCs were desensitised. This process continued as the experiment proceeded from 16% to 4% O_2, though at a slower rate.

The situation was the same whether the tissue responses were induced by 3μM NA (Figure 2) or 100mM KCl, (Figure 3). The extent of desensitisation when the tissue was activated with NA or depolarised with KCl are respectively shown in Figure 4a and b.

The introduction of Bay K 8644 into the saline before the second sequence of variation of oxygen (95%, 16% and 4%), temporarily interrupted the course of desensitisation, increasing the level of sensitivity for NA - induced responses. For potassium induced responses, only the first curve (for 95% 0_2) was at a higher level of sensitivity than the controls when Bay K 8644 was present in the physiological saline solution (Fig. 4b). Variation of oxygen tensions did not alter the continuous fall in the sensitivity of the tissue with time.

The presence of nifedipine during the third sequence resulted in a reduction of sensitivity to lower than control levels. For the NA – induced responses, there was a tendency for lower oxygen tension (4% and 16% O_2), to have a slightly

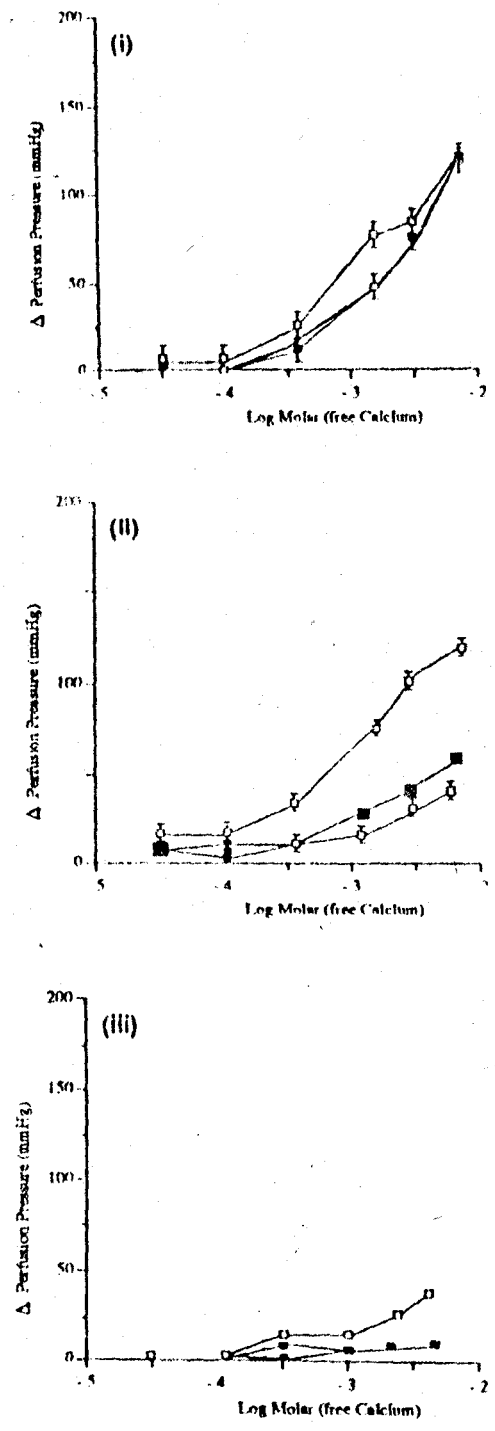

Fig. 3 *Sequential CCRCs constructed in three oxygen gas tensions of 95%, 16% and 4% in KCl - induced contractions of the rat tail artery (i) represents controls curves; (ii) is in the presence of Bay K8644 and (iii) is in the presence of nifedipine.*

higher level of sensitivity than at a higher oxygen tension (95% O_2), but the difference was not significant ($p>0.05$) (Fig. 4a). In contractions induced by KCL, variations of the oxygen tensions had virtually no effect on the influence of nifedipine on the level of sensitivity (Fig. 4b).

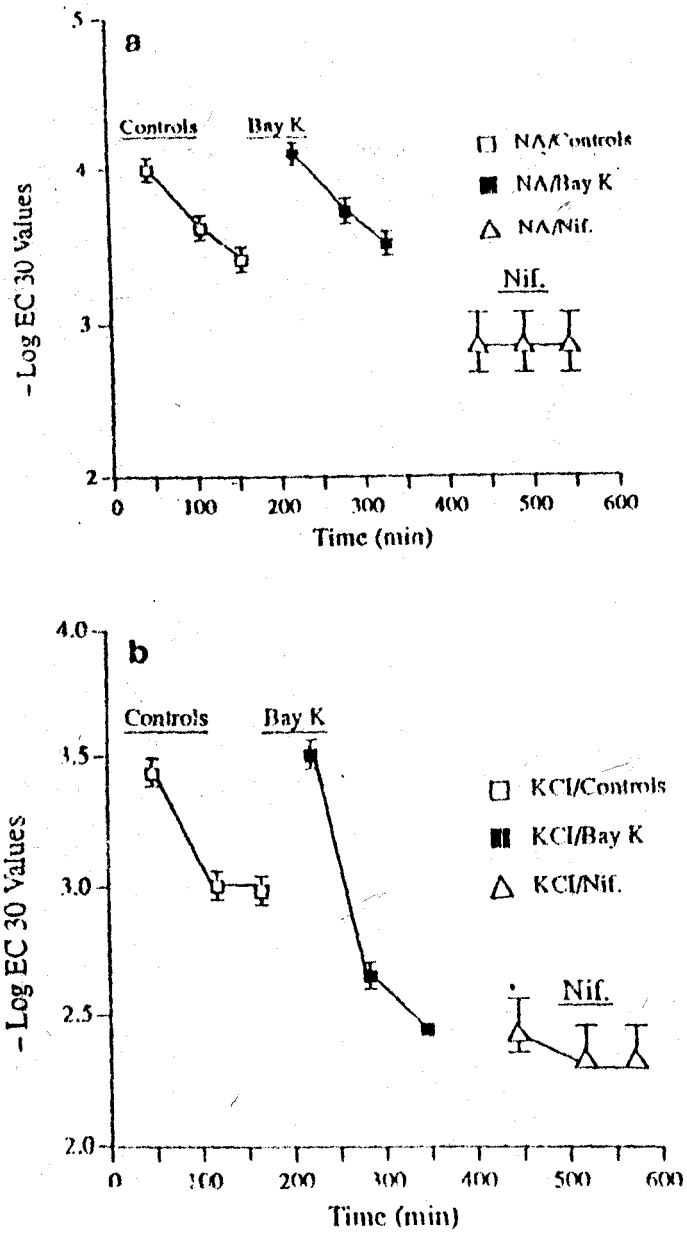

Fig. 4 *A plot of - log EC_{30} values against time showing the sensitivity of the tissue in decreasing levels of oxygen tension for control curves (□), Bay K8644 (■) and nifedipine (Δ) for NA-induced contractions (a) KCl - induced contractions (b).*

Discussion

It is clear from the results that while nifedipine shifted the curve to the right at 95% O_2, Bay K 8644 had no significant effect on the control curves. However, at lower oxygen tension of 16% O_2 and 4% O_2, Bay K 8644 shifted the curve leftwards. The effect of nifedipine under these conditions were less marked. When the shift in CCRCs at the level of 30% of the first control maximum response (EC_{30}) was plotted as the log ratio of EC_{30s} for Bay K 8644, control and nifedipine curves against O_2 tension, the influence of these drugs under the different oxygen tension was more clearly represented. The basis of the different effects at different gas tensions seem to be that the control curve shifts[8] (as in this logitudinal study at the different oxygen gas tensions in the same tissue).

The control curve shifted to the right but the curves in Bay K 8644 or nifedipine hardly changed as we decreased the oxygen from 95% O_2 to 16% to 4%. The most plausible interpretation

of this might be that the results indicate that the nifedipine - sensitive channels play a steadily decreasing role as the oxygen tension decreases but that they can be reactivated by Bay K 8644. It can therefore be inferred that the alteration of the effects of these drugs by mild hypoxia could be quite important in pathological hypoxia. This is because while nifedipine would be less likely to reverse noradrenaline-induced vasoconstriction in hypoxic conditions, Bay K 8644 is likely to intensify vasoconstriction.

In general, therefore, drugs which modify calcium channel function may, in vitro, be altered qualitatively and quantitatively by hyperoxic conditions, since oxygen appears to exert facilitatory effects on calcium channels.

With voltage - operated channels, it was surprising to note that at low partial pressures of oxygen, it was not possible to facilitate responses to KCl with Bay K 8644. On the contrary, Bay K 8644 reduced the tension. This is clearly demonstrated in Figure 4b, – where the shift in CCRC at the level of 30% of maximum response (EC_{30s}) for Bay K 8644, control and nifedipine against different oxygen tension. This observation is probably linked to the sequential procedures of longitudinal tests of the effects of Bay K 8644 starting with high oxygen tension and gradually decreasing the tension to lower values. It could therefore be concluded that when the tail artery is contracted with KCl, oxygen exerts a facilitatory influence on calcium channels. In addition, the effects of drugs that modify calcium channel function may be affected in magnitude and quality by the application of excess oxygen *in vitro*.

References

1. Detar R and Bohr DF: Oxygen and Smooth muscle contraction. Am. J. Physiol. 1968; 214, 241 – 244.
2. Egbeigbe AB, Pickard JD and Jenneth S: Responses of systemic vascular smooth muscle to hypoxia. Qrt. J. Exp. Physiol. 1980; 65: 273 –292.
3. Garry RC: The effect of oxygen lack on surviving smooth muscle. J. Physiol. 1928; 66: 235 – 248.
4. McGrath JC: Commentary: Evidence for more than one postjuctional - adrenoceptor. Biochem. Pharmac.1982; 31: 467–484.
5. McGrath JC: Adrenoceptor agonists and the Ca^{2+} - dependence of smooth muscle contraction; evidence for subtypes of receptors or for agonist-dependent differences in the agonist-receptor interaction? Clin. Sci., 1985; 68 (Suppl. 10). 55–635.
6. McGrath JC, Miller DJ and Smith GL: Contractile responses of rat anococcygeus smooth muscle to noradrenaline at buffered low levels of ionized calcium. J. Physiol. 1984; 353, 66P.
7. McGrath JC, Miller DJ and Ugwu AC: Factors affecting calcium sensitivity and the restoration of desensitised responses in the isolated perfused rat tail artery. J. Physiol. (Lond.), 1987; 392 46P.
8. Ugwu AC: Some factors that affect calcium - sensitivity in the rat tail artery. Ph.D. Thesis. University of Glasgow U.K. 1987a
9. Ugwu AC: Calcium-sensitivity and temperature effects on the desensitization of responses in different blood vessels isolated from the rat, rabbit and cat. J. Physiol. (Lond.), 1987b; 393 87P.