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Multiple effects of nordihydroguaiaretic acid on ionic currents in rat isolated type I carotid body cells

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1 The effects of the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) on the ionic currents of rat carotid body type I cells were investigated by use of whole-cell and outside-out patch clamp techniques.

2 NDGA (5–50 μM) produced a concentration-dependent inhibition of whole-cell K^+ currents at all activating test potentials (holding potential −70 mV). The time-course of the inhibition was also concentration-dependent and the effects of NDGA were only reversible following brief periods of exposure (<2 min). Another lipoxygenase inhibitor, phenidone (5 μM), was without effect on whole-cell K^+ currents in carotid body type I cells.

3 NDGA (5–50 μM) also inhibited whole-cell Ca^{2+} channel currents (recorded with Ba^{2+} as charge carrier) in a concentration-dependent manner.

4 Isolation of voltage-gated K^+ channels by use of high [Mg^{2+}] (6 mM), low [Ca^{2+}] (0.1 mM) solutions revealed a direct inhibition of the voltage-sensitive component of the whole-cell K^+ current by NDGA (50 μM).

5 In excised, outside-out patches NDGA (20–50 μM) increased large conductance, Ca^{2+} activated K^+ channel activity approximately 10 fold, an effect which could be reversed by either tetraethylammonium (10 mM) or charybdotoxin (30 nM).

6 It is concluded that NDGA activates maxi-K^+ channels in carotid body type I cells and over the same concentration range inhibits voltage-sensitive K^+ and Ca^{2+} channels. The inhibition of whole cell K^+ currents seen is most likely due to a combination of direct inhibition of the voltage-sensitive K^+ current and indirect inhibition of maxi-K^+ channel activity through blockade of Ca^{2+} channels.

Keywords: Carotid body; K^+ channels; Ca^{2+} channels; nordihydroguaiaretic acid; tetraethylammonium; charybdotoxin

Introduction

Over the past few years the ability of arachidonic acid (AA) and its various metabolites to modulate ion channel function has been the focus of much attention (Volterra *et al*., 1992; Chesnoy-Marchais & Fritsch, 1994; Yamada *et al*., 1994; Petit-Jacques & Hartzell, 1996; for reviews see Piomelli & Greengard, 1990; Jones & Persaud, 1993; Meves, 1994). One key enzyme family responsible for the production of biologically active AA metabolites is the lipoxygenase family (Piomelli & Greengard, 1990; Meves, 1994). The lipoxygenases are responsible for conversion of AA to the hydroxyperoxyeicosatetraenoic acids and the leukotrienes (Needleman *et al*., 1986; Piomelli & Greengard, 1990). Evidence to support the physiological role for such metabolites has frequently come from studies in which pharmacological inhibitors of AA metabolism have been employed, including nordihydroguaiaretic acid (NDGA). NDGA at low concentrations (<5 μM) is a relatively selective inhibitor of the lipoxygenases (Beetens *et al*., 1986), whilst at concentrations >5 μM NDGA can also inhibit cyclo-oxygenase (Beetens *et al*., 1986; Piomelli & Greengard, 1990; Safayhi *et al*., 1992) and phospholipase A_2 (Billah *et al*., 1985; Jacobson & Schrier, 1993).

NDGA (1–100 μM) has previously been shown to inhibit the generation of Ca^{2+}-dependent long term potentiation in neuronal cells (Williams & Bliss, 1988; Lynch *et al*., 1989). Over the same concentration range NDGA can also inhibit Ca^{2+}-dependent hormone secretion in pituitary cells, an effect which can be prevented by the Ca^{2+} ionophores A23187 and ionomycin (Luini & Axelrod, 1985; Chang *et al*., 1987). That NDGA prevented Ca^{2+} entry to cells via its effects on AA metabolism was cast into doubt by Korn & Horn (1990) who demonstrated inhibition of both transient and sustained voltage-gated Ca^{2+} channels in GH_3 and AtT-20 cells with NDGA, independently of effects on AA metabolism. Similarly, Wang *et al*. (1993) demonstrated direct inhibition of T-type Ca^{2+} channels by NDGA in mouse fibroblasts.

In light of these studies we have investigated the effects of NDGA on the ionic currents of carotid body type I cells by means of the patch-clamp technique. The carotid body is the principle arterial chemoreceptor which detects changes in arterial O_2, CO_2 and pH levels. In response to hypoxia, hypercapnia or acidosis, the carotid body initiates increased activity of chemosensory afferent fibres which project centrally and initiate corrective changes in breathing patterns (Gonzalez *et al*., 1994). Within the carotid body, type I cells are accepted as the chemosensory elements; these cells are neuroectodermal in origin and release transmitters in a Ca^{2+}-dependent manner in response to chemostimuli such as hypoxia and acidosis (Gonzalez *et al*., 1994; Peers & Buckler, 1995). These cells possess voltage-gated Ca^{2+} channels, Ca^{2+}-dependent K^+ channels and voltage-gated, delayed rectifier-like K^+ channels (Peers & Green, 1991; Peers & Buckler, 1995; Peers *et al*., 1996). Our results indicate that NDGA inhibits both Ca^{2+} channels and voltage-gated K^+ channels, but causes activation of Ca^{2+}-dependent K^+ channels.

Methods

Cell isolation

Isolation and culture of rat carotid body type I cells was essentially performed as previously described (Hatton & Peers, 1996). Briefly, 8–12 day old Wistar rat pups (4 or 5 per preparation) were anaesthetized by breathing 5% halothane, 95% oxygen through a face mask. Carotid bodies were removed and placed into phosphate-buffered saline containing 50 μM Ca^{2+},

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0.05% collagenase (Lorne Laboratories), and 0.020–0.025% trypsin (Sigma). Once all carotid bodies were removed, they were placed in a heated water bath (37°C) for 15 min, teased apart with fine forceps and then returned to the water bath for a further 3–4 min. The digested tissue was then triturated, centrifuged at 200 *g* for 5 min, resuspended in Ham's F-12 medium containing insulin (80 ul^{-1}), penicillin (100 iu ml^{-1}), streptomycin (100 $\mu g\ ml^{-1}$) and 10% foetal calf serum (GIBCO), centrifuged for a further 5 min, resuspended in Ham's once more and the isolated cell suspension was then placed onto a poly-D-lysine-coated coverslip. The cells were allowed to adhere for 2 h and were stored in a humidified incubator (37°C) for up to 30 h before being used for experiments.

Electrophysiology

Both single-channel and whole-cell patch clamp techniques were used to record ionic channel activity in type I cells (Hamill *et al*., 1981). On each experimental day pieces of coverslip were placed in a recording chamber (volume 80 μl) and continually perfused (at approximately 2 ml min^{-1}) with a standard extracellular solution composed of (in mM): NaCl 135, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2.5, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES) 5 and glucose 10 (pH 7.4, 21–24°C). In experiments to record currents through voltage-gated K^+ channels alone, without activation of Ca^{2+} dependent K^+ currents, $CaCl_2$ was reduced to 0.1 mM and $MgSO_4$ was raised to 6 mM, as demonstrated previously (Peers & Green, 1991; Hatton & Peers, 1996). For all whole-cell K^+ current recordings the pipette solution was of the following composition (in mM): KCl 117, $CaCl_2$ 1, $MgSO_4$ 2, NaCl 10, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA) 11, HEPES 11 and ATP 2 (pH 7.2), and electrodes were of 4–8MΩ resistance. Single K^+ channel recordings were made by use of excised outside-out patches exposed to the standard bathing solution, and pipettes with were filled with the same solution as for whole-cell recordings except that the added $CaCl_2$ was raised to 10.75 mM, calculated to yield a free [Ca^{2+}] of 10 μM (with CAMG software, W.H. Martin, Yale University). To record current flowing through Ca^{2+} channels in type I cells, the perfusate was composed of (in mM): NaCl 110, CsCl 5, $MgCl_2$ 0.6, $BaCl_2$ 10, HEPES 5, glucose 10 and tetraethylammonium 20 (pH 7.4, 21–24°C), and the patch pipettes contained (in mM): CsCl 130, EGTA 1.1, $MgCl_2$ 2, $CaCl_2$ 0.1, NaCl 10, HEPES 10 and ATP 2 (pH 7.2). NDGA (Sigma) and phenidone (Sigma) were freshly dissolved on each experimental day as 10 mM stock solutions in ethanol and dimethylsulphoxide (DMSO), respectively. These vehicles even at their highest concentrations (0.02% for ethanol, 0.002% for DMSO) were without effects on currents when applied alone (data not shown). Solution pH was adjusted to 7.4 where necessary after the addition of drugs.

For whole-cell recordings, cells were voltage-clamped at −70 mV and step depolarized (pulsed) to various test potentials (for construction of current-voltage relationships) or repeatedly to +20 mV (for K^+ currents) or 0 mV (for Ba^{2+} currents) for 50 ms at a frequency of 0.2 Hz. Evoked currents were filtered at 1 or 2 kHz and digitized at 5 kHz before storage on computer disk. All voltage pulse protocols and data acquisition were performed by use of the pCLAMP software suite (version 6.0.2) in combination with a Digidata 1200 interface board (Axon Instruments). Currents were measured for amplitude over the last 10–15 ms of the step depolarization, or at their peak if they showed any inactivation. Current inhibition was determined by expressing the current amplitude recorded in the presence of a drug as a percentage of control current amplitude, after leak subtraction. This was achieved by subtraction of appropriately scaled average currents evoked by small hyperpolarizing (10 mV) prepulses on-line (time-series experiments), or small hyperpolarizing ($\leqslant$30 mV) and depolarizing ($\geqslant$20 mV) voltage steps off-line (current-voltage relationships). Single-channel recordings were filtered at 0.2–2.0 kHz and digitized at 0.5–5 kHz again by use of the pCLAMP software suite.

Single channel analysis was performed off line by use of Fetchan software (Axon Instruments). Channel activity is expressed as NP_o (number of active channels in a patch x open probability) and was calculated by dividing mean current during control or drug application periods (with recording periods of 10–14 s) by the unitary current amplitude, which under these conditions was 9.2±0.1 pA (mean±s.e.mean from 7 patches), in good agreement with our previous study (Wyatt & Peers, 1995).

Results are presented as means±s.e.mean and statistical analyses were made by use of paired Student's *t* test, with $P<0.05$ being considered significant.

Results

Effects of NDGA on whole-cell K^+ currents

Bath application of NDGA (5–50 μM) caused a voltage-independent inhibition of whole-cell K^+ currents in isolated type I cells. This is exemplified in Figure 1a, which shows the mean current-voltage (*I*-V) relationships from 6 type I cells before, during and after exposure to NDGA. Note that current inhibition was observed at all activating test potentials and that

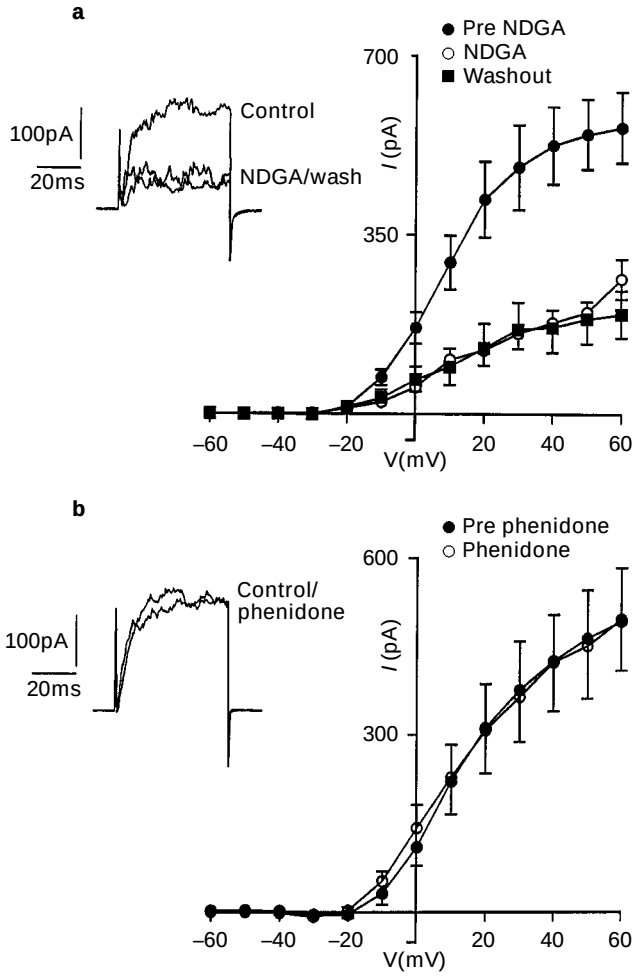

Figure 1 (a) Mean current-voltage (*I*-V) relationships (with vertical lines showing s.e.mean) obtained from 6 type I cells before application of 20 μM NDGA (Pre NDGA), during exposure to NDGA and following washout. Inset shows evoked currents from an example type I cell, all evoked by step depolarizations to +20 mV (holding potential −70 mV), under the conditions indicated. (b) Mean *I*-V relationships from 5 type I cells before and during bath application of 5 μM phenidone. Inset shows example currents evoked as in (a), under the conditions indicated.

little recovery of currents toward control values was seen after 6 min exposure. The inhibitory actions of NDGA are unlikely to be due to inhibition of lipoxygenase, since phenidone (5 μM) was without effect on *I*-V relationships recorded under identical conditions (Figure 1b).

Figure 2a shows a plot of the current inhibition versus concentration relationship for NDGA at equilibrium, either without (solid line) or with (dashed line) correction for current run-down. Correction for run-down was performed by simply recording evoked K^+ current amplitudes during repeated step depolarizations without additon of drug (solid squares, Figure 2b). The degree of current inhibition was then taken as the difference in mean current amplitude in untreated cells at the same time point as equilibrium was observed in the presence of different concentrations of NDGA. Clearly, NDGA produced concentration-dependent inhibitions of K^+ currents, with significant reductions in current amplitude being seen throughout the concentration range tested. The rate of inhibition of K^+ currents by NDGA also increased with increasing concentration, as exemplified in Figure 2b, equilibrium being reached within 2 min at 50 μM compared with over 10 min at 5 μM. Reversal of the inhibition by NDGA appeared to be time- rather than concentration-dependent: clear reversal of inhibition was observed on washout of 50 μM NDGA when applied for <2 min (e.g. Figure 2c), but when applied for longer periods, no recovery was observed, even at lower concentrations (e.g. Figure 2d).

Effects of NDGA on whole-cell Ca^{2+} channel currents

NDGA was tested over the same concentration range on Ca^{2+} channel currents recorded with 10 mM Ba^{2+} as the charge carrier. As with K^+ currents, NDGA potently inhibited Ca^{2+} channel currents (Figure 3). The time course of NDGA inhibition was again also concentration-dependent, more rapidly developing inhibition being seen at 50 μM (Figure 3a) than at 5 μM (Figure 3b). However, unlike for K^+ currents, the inhibition of Ca^{2+} channel currents was always at least partially reversible. Inhibition of Ca^{2+} channel currents was plotted against NDGA concentration (Figure 3d), and it is apparent that at the highest concentration tested (50 μM) NDGA caused almost complete inhibition of currents. Figure 3c illustrates the effects of 10 μM NDGA on Ca^{2+} channel currents recorded over a wide range of test potentials, and, as can be seen, the inhibition caused by NDGA (10 μM) showed no obvious voltage-dependence, strong inhibition being detected at all activating test potentials (Figure 3c, representative of 5 cells tested).

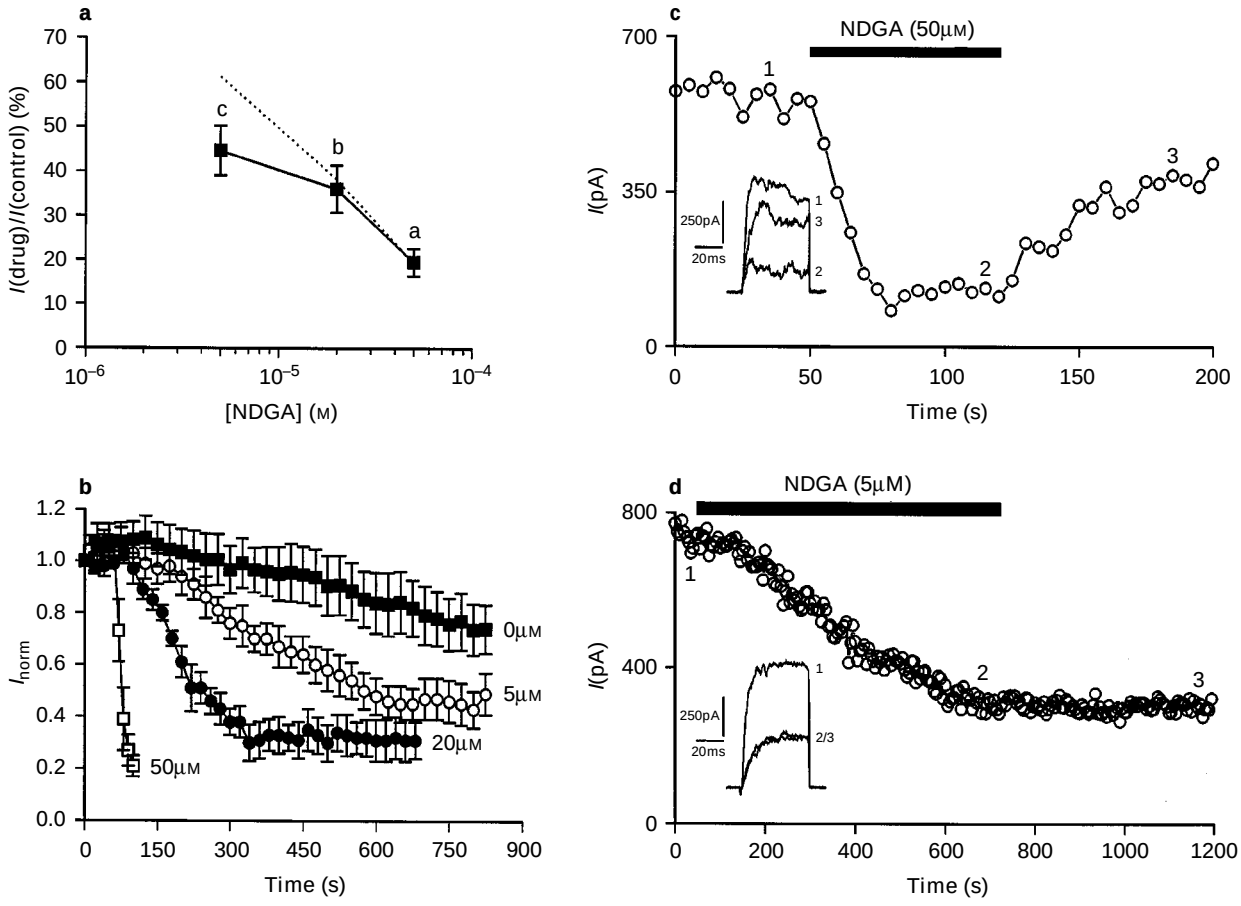

Figure 2 (a) Concentration-response relationship for NDGA acting on whole cell K^+ currents. Significant reductions caused by NDGA (paired Student's *t* test):[a]$P<0.0003$; [b]$P<0.004$; [c]$P<0.03$. Each point plotted is the mean from 4–6 cells in each case; vertical lines show s.e.mean. The dashed line indicates the concentration-response relationship for NDGA when correction for current run-down with time was made (see text for further details). (b) Normalized mean time series plots, from the same cells as used to construct (a), where each plotted point is mean (and s.e.mean) K^+ current amplitude evoked by successive step depolarizations from -70 to $+20$ mV (0.2 Hz, 50 ms). In each case cells were exposed to NDGA from time 0, and throughout the period plotted. Cells were exposed to either 5 μM, 20 μM or 50 μM NDGA, or were recorded in the absence of NDGA 0μM. (c) Time-series plot from an example type I cell exposed to 50 μM NDGA. Each point plotted is K^+ current evoked by successive depolarizations (50 ms duration, 0.2 Hz) to $+20$ mV (holding potential -70 mV). Period of exposure to NDGA is indicated by the solid horizontal bar. (d) Time series plot taken from another type I cell under the same conditions as (c), but in this case 5 μM NDGA was used. Solid horizontal bar indicates the period of exposure to NDGA. Inset K^+ currents in (c) and (d) are examples corresponding to the numbered points shown in each plot.

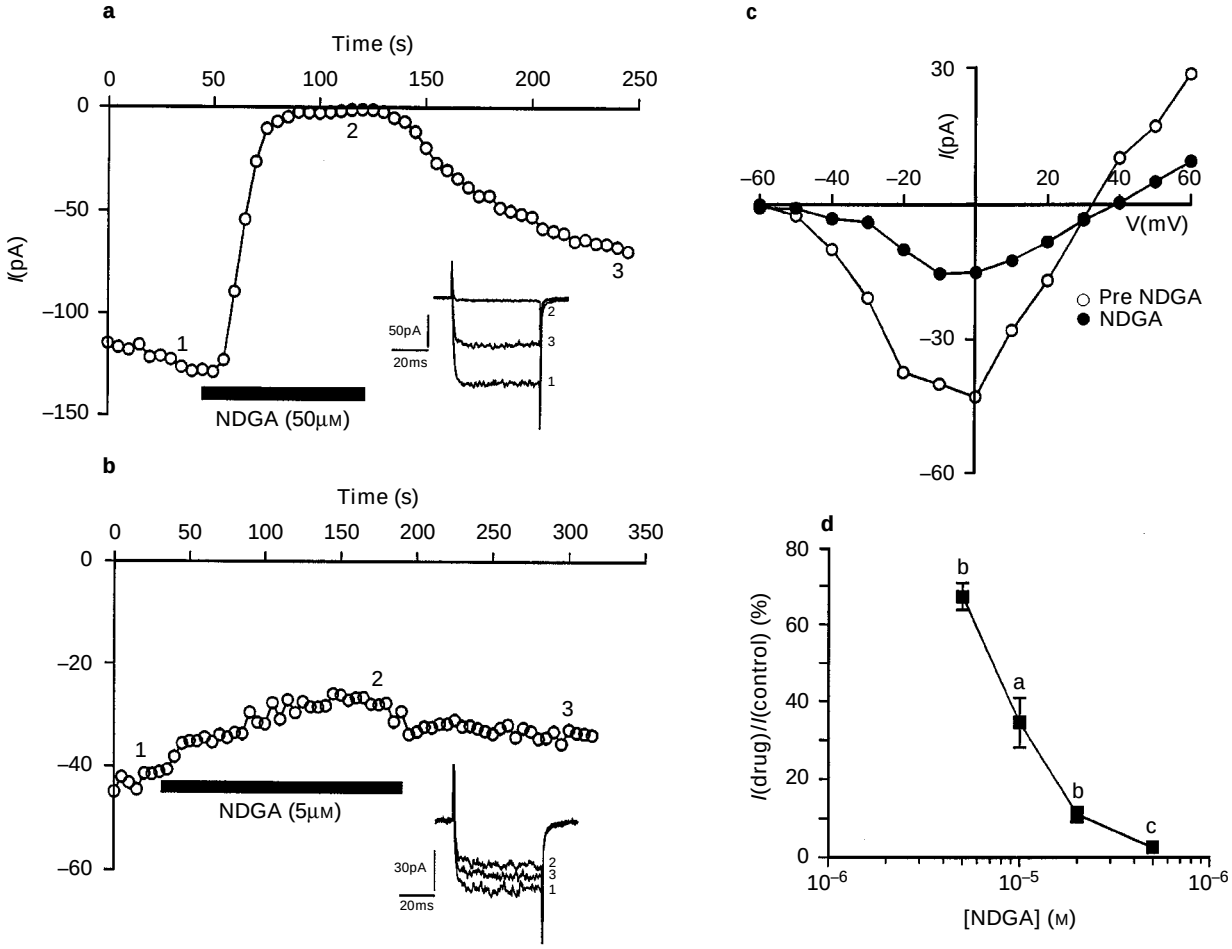

Figure 3 (a) Time series plot from an example type I cell exposed to 50 μM NDGA. Each point is amplitude of Ba^{2+} current evoked by successive depolarizations from -70 to 0 mV (50 ms, 0.2 Hz). Period of NDGA exposure is indicated by the solid horizontal bar. (b) As (a), but for another type I cell exposed to 5 μM NDGA for the period indicated by the solid horizontal bar. Insets in (a) and (b) show example Ca^{2+} channel currents for corresponding time-series plots, each current corresponding to the numbered point on the adjacent time-series plot. (c) Ba^{2+} current-voltage relationship obtained from a representative type I cell before and during exposure of the cell to 10 μM NDGA. (d) Concentration-response relationship for NDGA acting on whole cell Ca^{2+} channel currents, 10 mM Ba^{2+} as charge carrier. Significant reductions caused by NDGA (paired Student's t test): $^{a}P<0.006$; $^{b}P<0.006$; $^{c}P<0.05$. Each point is plotted as mean taken from 4–6 cells in each case; vertical lines show s.e.mean.

Inhibition of Ca^{2+}-insensitive K^+ currents

Under the experimental conditions used for recording whole cell K^+ currents in type I cells, the activity of Ca^{2+}-dependent K^+ currents is dependent on Ca^{2+} influx via Ca^{2+} channels during step depolarizations (see e.g. Peers & Green, 1991). Inhibition of Ca^{2+} channel currents by NDGA (Figure 3) may therefore account, at least in part, for the observed inhibition of whole-cell K^+ currents. The Ca^{2+}-dependent K^+ currents account for approximately half of the whole-cell K^+ current in type I cells, the remainder being attributable to Ca^{2+}-insensitive, delayed rectifier-like K^+ channels (Peers, 1990a). To investigate whether NDGA could inhibit this latter component of current, we used a high [Mg^{2+}] (6 mM), low [Ca^{2+}] (0.1 mM) perfusate to prevent Ca^{2+} influx and hence significant activation of Ca^{2+}-dependent K^+ currents (Peers & Green, 1991; Hatton & Peers, 1996). Under these conditions NDGA (50 μM) potently inhibited the residual Ca^{2+}-insensitive K^+ current, as exemplified in Figure 4. In 5 cells tested, the degree of inhibition was $82.7\pm4.0\%$.

Close inspection of the example traces in Figure 4 shows that, under normal conditions, K^+ currents are noisy (see also Figure 1a), due to the activity of Ca^{2+}-dependent K^+ channels which are of high (approximately 200pS) conductance (Wyatt & Peers, 1995). When the high [Mg^{2+}], low [Ca^{2+}] perfusate was applied, currents were reduced in amplitude and also far less noisy (trace 2, Figure 4), due to inhibition of high conductance K^+ channels. However, when NDGA was applied, despite currents being reduced in amplitude still further, there was a clear increase in current noise (right trace, Figure 4) similar to that seen under control conditions. Although not investigated further by whole-cell recordings, this observation raised the possibility that NDGA might activate Ca^{2+}-dependent K^+ channels, and we therefore used single channel recordings to investigate this further.

Effects of NDGA on Ca^{2+}-dependent K^+ channels

Ca^{2+}-dependent K^+ channel activity was recorded in outside-out patches with 10 μM Ca^{2+} in the pipette to augment channel activity. Under these conditions Ca^{2+}-dependent K^+ channel openings could readily be determined by their amplitude (9.2 ± 0.1 pA, $n=9$ patches recorded at a membrane potential of +20 mV) compared to previous recent studies from this laboratory (Wyatt & Peers, 1995; Hatton & Peers, 1996). Figure 5a shows an example recording of 1 of 9 such patches in which brief exposure to 20 μM NDGA always reversibly increased activity of these high conductance channels. Mean channel activity (NP_o) increased from 0.36 ± 0.26 under control conditions to 3.74 ± 1.02 in the presence of NDGA ($n=9$). Also illustrated in Figure 5 is the observation that when the perfusate containing NDGA was exchanged for one contain-

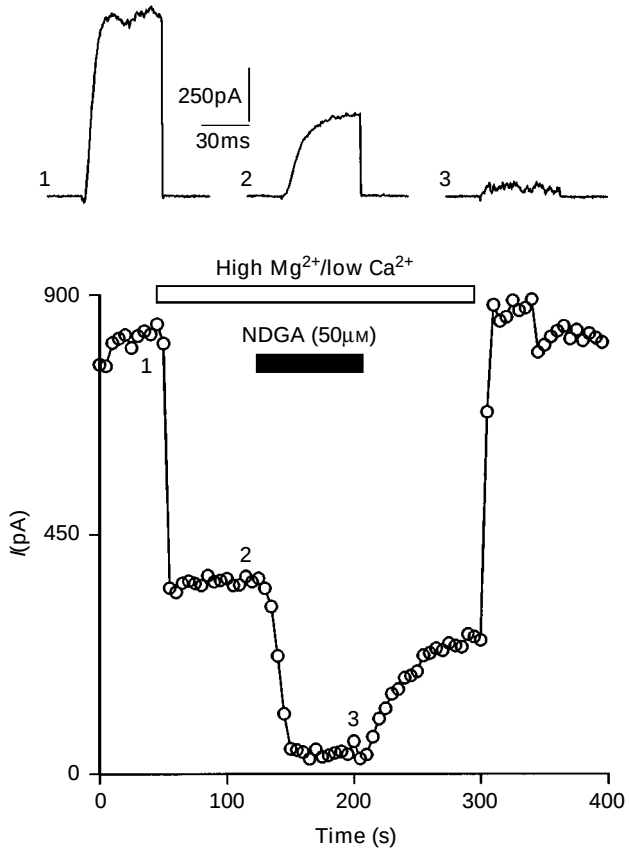

Figure 4 Example time-series plot in which each point is a K^+ current amplitude evoked by successive step depolarizations to +20 mV (50 ms, 0.2 Hz, holding potential −70 mV). Open horizontal bar indicates period of exposure to perfusate containing 6 mM Mg^{2+} and 0.1 mM Ca^{2+} (standard perfusate 1.2 mM Mg^{2+}, 2.5 mM Ca^{2+}). Period of exposure to 50 μM NDGA indicated by solid horizontal bar. Above, K^+ currents corresponding to the numbered points on the time series plot.

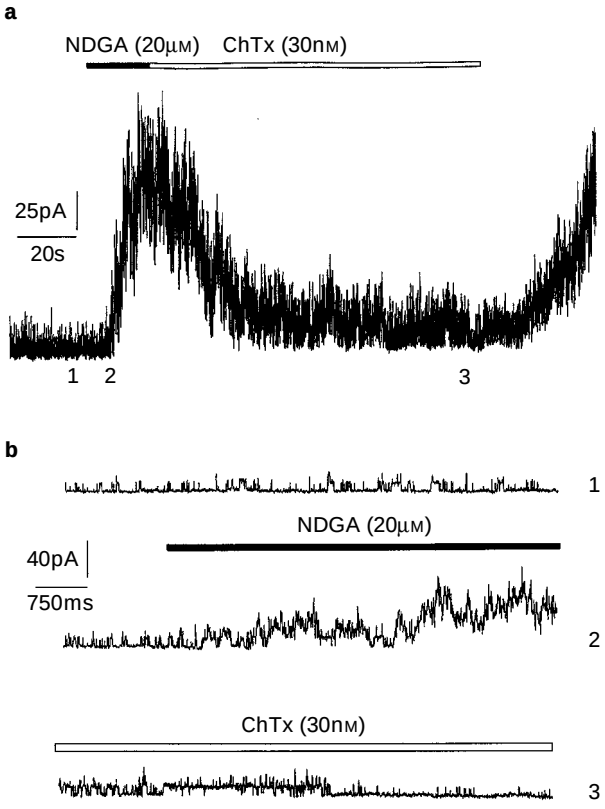

Figure 5 (a) Representative recording from an excised, outside-out patch containing several large conductance Ca^{2+}-activated K^+ channels. Channel openings are upward deflections. Pipette potential +20 mV. Solid bar indicates periods of exposure to 20 μM NDGA, open bar indicates periods of exposure to 30 nM charybdotoxin. (b) Example traces shown on an expanded time scale. Each numbered trace corresponds to the numbered point in (a). Again, solid horizontal bar indicates period of exposure to 20 μM NDGA, open bar indicates period of exposure to 30 nM charybdotoxin. Calibration bars apply to all 3 traces.

ing 30 nM charybdotoxin, NP_o was dramatically reduced, to 1.04 ± 0.67 ($n = 5$ patches), indicating that NDGA was indeed activating high conductance, charybdotoxin-sensitive channels which underlie the Ca^{2+}-dependent component of the whole-cell K^+ current. On washout of charybdotoxin (but without re-addition of NDGA) channel activity once more increased (Figure 5), indicating the poor reversibility of this compound. When tetraethyammonium (10 mM) was used in place of charybdotoxin, this always abolished channel activity ($n = 4$, data not shown). Figure 5b shows sections taken from the above trace in control, NDGA and charybdotoxin-containing solutions on an expanded time-scale. Exposure of patches to higher concentrations of NDGA (50 μM) always produced an initial burst of channel openings which was rapidly followed by a disruption of the membrane and loss of the patch ($n = 3$, data not shown).

Discussion

NDGA is commonly used as a lipoxygenase inhibitor. However, recent studies from Korn & Horn (1990) have shown that this compound can produce profound inhibitory effects on Ca^{2+} channels of various cell types at concentrations used in the study of AA metabolism. In our hands, NDGA inhibited Ca^{2+} channel currents in carotid body type I cells over a similar concentration range (5–50 μM), in good agreement with the above study. Ca^{2+} channels are heterogeneously distributed in type I cells of the rat carotid body: whilst all cells display dihydropyridine sensitivity, indicative of the presence of L-type Ca^{2+} channels, the degree of inhibition of Ca^{2+} channel currents by supramaximal nifedipine concentrations varies markedly from cell to cell (Peers *et al.*, 1996). Furthermore, a minority of cells display sensitivity to inhibition by ω-conotoxin GVIA (indicative of the presence of N-type channels), but the majority of cells are insensitive to this toxin (Peers *et al.*, 1996). Furthermore, on the basis of such pharmacological studies, there is a non-L, non-N type component of the Ca^{2+} current in type I cells (e Silva & Lewis, 1995; Peers *et al.*, 1996). We cannot at present know if NDGA shows any selectivity for particular Ca^{2+} channel subtypes in our cells, but the fact that at high concentrations (50 μM) NDGA inhibited virtually all the Ca^{2+} current suggests that this agent is a non-selective Ca^{2+} channel inhibitor.

In the present study we demonstrated inhibition of K^+ currents by NDGA. Separation of two different components of the whole-cell K^+ currents uncovered two distinct effects of NDGA in carotid body type I cells. Inhibition of whole-cell K^+ currents can occur indirectly via blockade of Ca^{2+} channels, which prevents activation of the Ca^{2+}-dependent K^+ channels which account for approximately half of the whole-cell K^+ current (Peers, 1990). Because NDGA is a known Ca^{2+} channel inhibitor (Korn & Horn, 1990; Wang *et al.*, 1993), and since we have demonstrated inhibition of Ca^{2+} channels in carotid body type I cells in the present study (Figure 3), it is probable that at least part of the inhibition of the whole-cell K^+ current is through this indirect mechanism. However, NDGA was able to inhibit >80% of the whole-cell K^+ current, more than can be

accounted for by indirect inhibition of the Ca^{2+} sensitive component of the K^+ current. This implies a direct inhibitory effect of NDGA on the voltage-gated component of the whole-cell K^+ current. By use of a modified perfusate containing high [Mg^{2+}] (6 mM) and low [Ca^{2+}] (0.1 mM), the threshold for activation of the Ca^{2+} sensitive component is shifted markedly to more positive test potentials by preventing Ca^{2+} influx, thus preventing significant activation over the voltage range tested, allowing us to study the voltage-sensitive component of K^+ current in isolation (Peers & Green, 1991; Hatton & Peers, 1996). Under these conditions a direct effect of NDGA on the voltage-gated K^+ current was apparent. Indeed, 50 μM NDGA virtually abolished K^+ currents under these conditions. Interestingly, the small residual currents seen in the presence NDGA in these experiments were strikingly noisy, suggesting perhaps a direct activation of high conductance Ca^{2+}-dependent K^+ channels by NDGA, as has been recently described by Nagano *et al*. (1996) in porcine aortic smooth muscle cells. By use of outside-out patches to prevent effects due to blockade of Ca^{2+} channels, a potent stimulant effect of NDGA on Ca^{2+}-dependent K^+ channels was indeed seen (Figure 5): 20 μM NDGA increased channel activity approximately 10 fold in every case. NDGA therefore directly stimulates Ca^{2+}-dependent K^+ channel activity in carotid body type I cells. Interestingly, GH_3 cells are known to possess Ca^{2+}-dependent K^+ channels (Ritchie, 1987). However, Korn & Horn (1990) did not investigate the effects of NDGA on K^+ currents in their study. In type I cells, 50 μM NDGA again produced an initial large increase in channel activity in outside-out patches, but this was accompanied by a disruption of the membrane and loss of the patch. This detergent like effect of high (>30 μM) concentrations of NDGA was also noted by Korn & Horn (1990). This observation, together with the relatively slow onset of action of NDGA and the time-dependence of its reversibility are suggestive of a compound which partitions into the membrane to produce its actions. This hypothesis is supported by the observation of Korn & Horn (1990) that NDGA does not require direct access to the extracellular surface of Ca^{2+} channels in GH_3 and AtT-20 cells to cause inhibition. Similarly, Nagano *et al*. (1996) observed increased Ca^{2+}-dependent K^+ channel activity following bath application of NDGA when both the inside-out and outside-out patch configurations were used. The lipophilic nature of NDGA is likely to account also for the fact that the degree of reversibility of NDGA inhibition was time- rather than concentration-dependent. Following brief exposure (>2 min) to NDGA recovery of K^+ channel amplitudes on wash was almost complete, whilst after longer periods of exposure the effects of NDGA appeared to be irreversible, and again this is compatible with the observations of Korn & Horn (1990).

That the actions of NDGA are not a consequence of lipoxygenase inhibition is supported by the observation that phenidone, a lipoxygenase inhibitor which is structurally unrelated to NDGA, was unable to mimic the effects of NDGA in carotid body type I cells (Figure 1b). Korn & Horn (1990) tested a range of lipoxygenase inhibitors for NDGA-like activity in GH_3 and AtT-20 cells and found only ketoconazole was additionally able to inhibit Ca^{2+} channels. This observation is perhaps not surprising since we have previously demonstrated direct inhibition of both K^+ and Ca^{2+} channels in carotid body type I cells by the related imidazole antimycotics miconazole and clotrimazole in the low μM range (Hatton & Peers, 1996). Furthermore, activation of Ca^{2+}-dependent K^+ channels was demonstrated in excised outside-out patches (Figure 5; see also Nagano *et al*., 1996): since lipoxygenase is a soluble cytosolic enzyme, and is not active if it becomes membrane-bound (Rouzar & Kargman, 1988; Rouzar *et al*., 1990), it would appear to be a reasonable assumption that lipoxygenase activity was not present in excised outside-out patches.

This profile of inhibition of Ca^{2+} channels, but activation of the Ca^{2+}-dependent K^+ channels by NDGA shows a remarkable similarity to that of the benzimidazole Ca^{2+}-dependent K^+ channel openers NS 004 and NS 1619 (Sargent *et al*., 1993; Edwards *et al*., 1994). In a similar manner to NDGA, NS 1619 (10–33 μM) was shown to inhibit Ca^{2+} and delayed rectifier K^+ channels in rat portal vein smooth muscle cells, whilst over the same concentration range NS 1619 was able to induce a charybdotoxin-sensitive K^+ current from a holding potential of -10 mV (Edwards *et al*., 1994). The striking similarity of the pharmacological profile of NDGA to that of the benzimidazole Ca^{2+}-dependent K^+ channel openers typified by NS 004 and NS 1619 (Sargent *et al*., 1993; Edwards *et al*., 1994) suggets that NDGA could perhaps form the basis for the development of a new class of Ca^{2+}-dependent K^+ channel opening agents. At the very least the observation that NDGA can produce multiple profound effects on the ionic currents of carotid body type I cells requires that caution be exercised when effects of NDGA on arachidonic acid metabolism are interpreted.

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