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Nitric Oxide Inhibition of the Rat Olfactory Cyclic Nucleotide-Gated Cation Channel

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Abstract. The effects of nitric oxide (NO) and other cysteine modifying agents were examined on cyclic nucleotide-gated (CNG) cation channels from rat olfactory receptor neurons. The NO compounds, S-nitroso-cysteine (SNC) and 3-morpholino-sydnonomine (SIN-1), did not activate the channels when applied for up to 10 min. The cysteine alkylating agent, N-ethylmaleimide (NEM), and the oxidising agent, dithionitrobensoate (DTNB), were also without agonist efficacy. Neither SNC nor DTNB altered the cAMP sensitivity of the channels. However, 2-min applications of SIN-1, SNC and DTNB inhibited the cAMP-gated current to approximately 50% of the control current level. This inhibition showed no spontaneous reversal for 5 min but was completely reversed by a 2-min exposure to DTT. The presence of cAMP protected the channels against NO-induced inhibition. These results indicate that inhibition is caused by S-nitrosylation of neighboring sulfhydryl groups leading to sulfhydryl bond formation. This reaction is favored in the closed channel state. Since recombinantly expressed rat olfactory α and β CNG channel homomers and α/β heteromers are activated and not inhibited by cysteine modification, the results of this study imply the existence of a novel subunit or tightly bound factor which dominates the effect of cysteine modification in the native channels. As CNG channels provide a pathway for calcum influx, the results may also have important implications for the physiological role of NO in mammalian olfactory receptor neurons.

Key words: Cyclic nucleotide-gated ion channel — Rat olfactory receptor neuron — Nitric oxide — Inhibition — Sulfhydryl binding agent

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Introduction

The detection of odorants by olfactory receptor neurons is mediated by the activation of G protein-coupled receptors which leads to a rapid increase in the intracellular concentration of cAMP. Subsequent direct activation by cAMP of the olfactory cyclic nucleotide-gated (CNG) cation channel results in cation influx and neuronal excitation. CNG channels have now been shown to have a widespread distribution in neuronal and non-neuronal tissues (Kaupp, 1995; Finn, Grunwald & Yau, 1996). In general, they form a crucial link between the stimulus-induced chemical change and the electrical signal that is transmitted throughout the nervous system.

Since the cloning of the first CNG subunit from the bovine photoreceptor (Kaupp et al., 1989), it has been apparent that members of this family share a strong structural resemblance with members of the voltage-gated ion channel superfamily (Jan & Jan, 1990). Like voltage-gated ion channels, functional CNG channels can be formed from 4 subunits arranged in a ring to form a central ion-conducting pore (Liu, Tibbs & Siegalbaum, 1996). To date, two olfactory CNG subunits have been cloned from rat olfactory neuroepithelial cDNA libraries: an α subunit (Dhallan et al., 1990) and a β subunit (Liman & Buck, 1994; Bradley et al., 1994). Although functional expression of the α subunit alone forms cyclic nucleotide-activated channels, coexpression with the β subunit yields channels whose properties resemble more closely those of the native rat channel (Liman & Buck, 1994; Bradley et al., 1994). However, it remains to be established whether the recombinant α/β subunits are structurally and functionally identical to the native channel.

Recently, NO and other sulfhydryl modifying agents have been shown to act as potent agonists of both the salamander olfactory CNG channel and of recombinantly expressed rat α and β olfactory CNG channel subunits

(Broillet & Firestein, 1996*a*, 1997). Apart from the possible physiological importance of these observations, the identification of a novel ligand offers a possible means of discriminating between CNG channel isoforms. Accordingly, the main aim of this study is to determine whether NO and sulfhydryl modifying compounds can be used to functionally discriminate between the native rat CNG channel and the recombinant rat CNG channel subunits. The main outcome is that NO exerts a potent inhibitory effect on the native channel, which is contrary to its activating effect on every other CNG channel isoform studied to date. This provides evidence for a structural difference between the rat native channel and the recombinant rat α and β subunits. Furthermore, since CNG channels provide an entry pathway for calcium into these cells (Frings et al., 1995), the results may also have important physiological significance.

Materials and Methods

Olfactory receptor neurons were isolated from adult rats as previously described (Lynch & Barry, 1992). Dissociated cells were continuously superfused by a modified Tyrode's solution containing (in mM): NaCl 140, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, glucose 10, and HEPES 10 (pH 7.4 with NaOH). Membrane currents were recorded using standard patch-clamp techniques and all experiments were performed at room temperature (18–25°C). Patch pipettes, fabricated from borosilicate haematocrit tubing, had tip residences of between 8–12 MΩ when filled with the standard divalent cation-free pipette solution containing (in mM): NaCl 140, EGTA 10, HEPES 10, (pH 7.4 with NaOH). Olfactory receptor neurons were readily identified by their distinct bipolar shape. Cell-attached membrane patches were formed amongst the cilia on the apical knobs of these cells and detached into the inside-out configuration by brief air exposure. Excised patches were exposed to a low divalent cation solution containing (in mM): NaCl 140, EGTA 0.25, HEPES 10 (pH 7.4 with NaOH). Unless otherwise indicated, this was the standard control solution used in all experimental procedures. The '200 μM Ca^{2+}' solution used to assay the presence of calcium binding factor contained (in mM): NaCl 140, $CaCl_2$ 0.2, HEPES 10 (pH 7.4 with NaOH). The 'Mg-EGTA' solution used to ablate the calcium binding factor from the channel contained (in mM): NaCl 140, $MgCl_2$ 2, EGTA 10, HEPES 10 (pH 7.4 with NaOH). Excised patches were voltage-clamped at 0 mV and macroscopic patch currents were measured in response to voltage steps of 120 msec duration from −60 to +60 mV in 10-mV steps. Currents were recorded using an Axopatch 200B patch-clamp amplifier (Axon Instruments, Foster City, CA), filtered at 1 kHz and digitized at 2.5 kHz directly to disk. Experimental data are expressed as means ± one standard error of the mean (SEM). Statistical significance was assessed using a paired *t*-test, with a P value < 0.025 being considered significant. SNC was prepared as described previously (Lei et al., 1992; Broillet & Firestein, 1996*a*). Briefly, sodium nitrite and L-cysteine hydrochloride were combined in distilled water at a concentration of 100 mM and vortexed until completely dissolved and the solution turned a clear red color. A fresh batch of SNC was mixed after the formation of each patch to ensure that it retained maximum activity for the duration of each recording. In experiments lasting longer than 30 min, fresh batches of SNC were prepared at 30-min intervals. In control experiments, the original inhibitory efficacy of SNC was retained for at least 30 min after mixing but declined dramatically between 30 and 60 min. Depleted SNC, mixed more than 60 min previously, was frequently applied as a control and was never observed to elicit any response. SIN-1, NEM and DTNB were dissolved in DMSO at a concentration of 20 mM and were either used immediately or stored at −20°C for up to one month without any obvious deterioration in efficacy. The DTT solution was prepared freshly on each experimental day. All reagents were obtained from Sigma (St. Louis, MO). None of these agents caused any change in the pH of the perfusing solution at the concentrations used in this study.

Results

Sulfhydryl Modifying Agents Have No Agonist Efficacy

Activation of the rat olfactory CNG channel by NO and other sulfhydryl modifying agents was investigated in inside-out patches excised from the dendritic knob of adult rat olfactory receptor neurons. Such patches usually contain several hundred CNG channels (Frings, Lynch & Lindemann, 1992). Examples of macroscopic currents recorded in one patch before and after the addition of a saturating (100 μM) concentration cAMP are shown in Fig. 1*A* and *B*, respectively. Fig. 1*E* displays the net cAMP-gated current remaining after digital subtraction of the background current. Since this patch had a net cAMP-gated conductance of 5.33 nS and the unitary conductance of these channels is about 15 pS (Frings et al., 1992; Chiu, Lynch & Barry, 1997), it contained approximately 355 active channels.

Despite this large number of active channels, a 5-min application of the NO-liberating compound, S-nitroso-cysteine (SNC), did not induce any significant increase in the background leakage conductance when applied at a concentration of 1 mM (Fig. 1*C*). Net background conductances before, during and after the application of SNC were identical (Fig. 1*A*, *C* and *D*). Indeed, digital subtraction of the background currents (Fig. 1*A*) from currents recorded at corresponding voltages following SNC exposure (Fig. 1*C*) reveal no residual channel activation (Fig. 1*F*). Similar results were observed in each of 5 patches exposed to 1 mM SNC for 10 min and in 13 other patches exposed to 1 mM SNC for periods of 3–5 min. A lower (50 μM) SNC concentration was also without effect when applied for a period of 10 min in each of 3 patches. These observations contrast directly with those from the salamander olfactory CNG channel, where 100 μM SNC had an even greater agonist efficacy than did a saturating concentration of cAMP (Broillet & Firestein, 1996*a*). SNC was also a potent agonist of the recombinantly expressed rat α and β homomeric CNG channels and the rat α/β heteromeric channel (Broillet & Firestein, 1997). However, their claim that SNC activates the native rat olfactory CNG channel is unconvincing since the cAMP-sensitivity of the activated channels was not demonstrated (*see* Fig. 1*C* in Broillet & Fires-

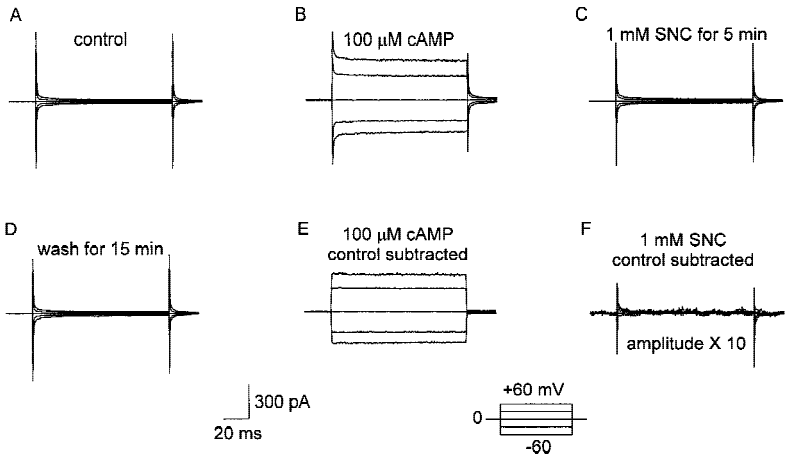

Fig. 1. SNC does not activate cAMP-gated channels in inside-out, multichannel patches from dissociated rat olfactory recepetor neurons. Data were recorded sequentially from *A–D* in the same patch. This patch contained about 355 active cAMP-gated channels. *(A)* Total (i.e., unsubtracted) currents activated by voltage steps from 0 mV to −60, −30, 0, 30, and 60 mV. This voltage protocol, summarized in the inset, applies to all currents displayed in Figs. 1–5. *(B)* Total current responses in the same patch following application of 100 μM cAMP. *(C)* Total current responses recorded after a 5-min application of 1 mM SNC. *(D)* Total current responses recorded following a 15-min wash in control solution. *(E)* Net cAMP-gated currents, obtained by subtracting the current traces in *A*, from the corresponding currents in *B*. All subsequent cAMP-gated currents in this paper are displayed as such difference currents. *(F)* Net current activated by 1 mM SNC, obtained by subtracting the average of *A* + *D* from *C*. The scale bars apply to all traces except *F*, where the current amplitude has been magnified by a factor of 10 to emphasize the complete lack of activation.

tein, 1997). The agonist efficacy of several other cysteine modifying reagents was also examined. As shown in the example in Fig. 2*A*, the NO donor, 3-morpholino-syndronomine (SIN-1), had no detectable effect when applied at a concentration of 100 μM for either 3–5 min ($n = 13$ patches) or for up to 10 min ($n = 3$ patches). Similarly, the sulfhydryl alkylating agent, N-ethylmaleimide (NEM), showed no measurable agonist efficacy when applied at a concentration of 100 μM for either 3–5 min ($n = 9$ patches) or 10 min ($n = 2$ patches). An example of the result of a 3-min application of 100 μM NEM in one patch is shown is Fig. 2*B*. Finally, the oxidizing agent, dithionitrobenzoate (DTNB), had no agonist efficacy when applied at a concentration of 200 μM for 10 min in each of 3 patches or when applied for 3–5 min in each of 9 patches (e.g., Fig. 2*C*) In contrast, each of these compounds was a potent agonist of the salamander olfactory CNG channel (Broillet & Firestein, 1996*a*).

It is possible that the inability of these agents to activate the channel was due to the sulfhydryl groups being already covalently modified. This possibility was examined by applying a 2 mM concentration of the reducing agent, dithiotreitol (DTT), to the intracellular patch surface. When applied for a period of 2 min, DTT should break exposed disulfide bonds or molecular groups covalently linked to exposed sulfhydryl groups. However, DTT treatment had no effect on magnitude of the cAMP-gated conductance in freshly excised patches. In a total of 10 patches where DTT was applied to excised patches prior to treatment with sulfhydryl-binding agents, the averaged maximum cAMP-gated conductance was 98 ± 3% of the control current magnitude. This difference was not significant using a paired *t*-test. Furthermore, the 2-min DTT treatment had no effect on the susceptibility of the channels to activation by any of the above sulfhydryl modifying agents when each of the above agents was applied for up to 10 min in a total of 23 patches. Another possibility is that a tightly bound intracellular factor occludes one or more critical sulfhydryl groups. The only factor known to bind irreversibly to these channels in vivo is the 'calcium binding factor' described by Balasubramanian, Lynch and Barry (1996).

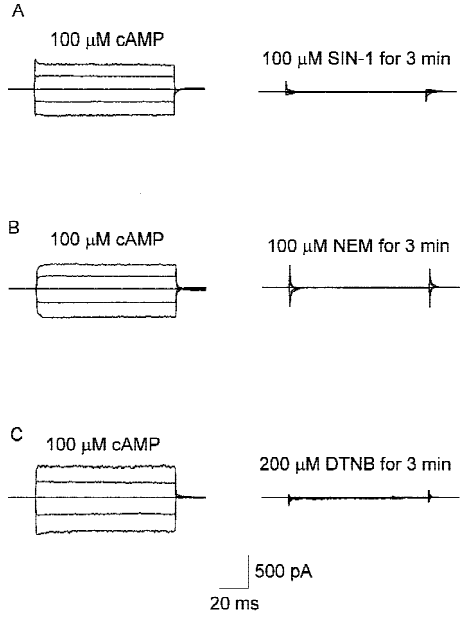

Fig. 2. Other sulfhydryl binding agents do not significantly activate the channel. *(A)* Although this patch contained about 409 active cAMP-gated channels (left panel), SIN-1 had no effect when applied for 3 min (right panel). *(B)* A 3-min application of 100 μM NEM had no effect in a patch which contained about 400 channels. *(C)* Although this patch contained 580 channels, a 3-min application of DTNB induced no significant activation. The scale bars apply to all displayed traces.

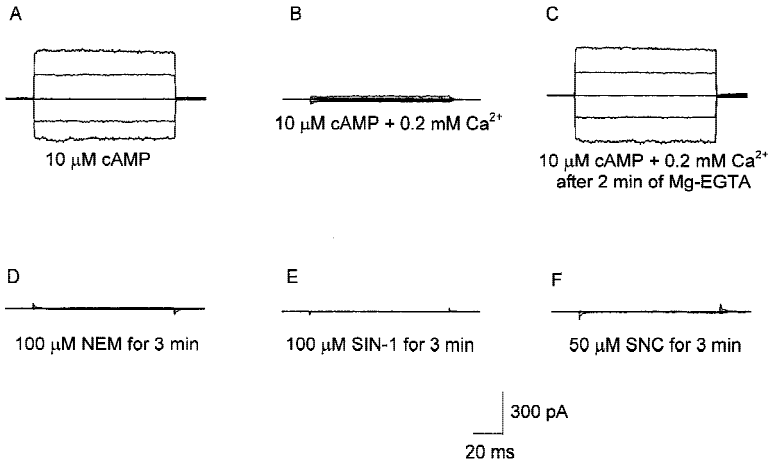

Fig. 3. Ablation of the calcium binding factor has no effect on the ability of sulfhydryl binding agents to activate the channel. All data were recorded sequentially from *A–E* in the same patch. This patch contained about 392 active cAMP-gated channels. *(A)* Activation of CNG channels by an almost saturating (10 μM) concentration of cAMP. *(B)* Application of 10 μM cAMP + 0.2 mM calcium inhibits the CNG current. *(C)* Currents recorded in the presence of 10 μM cAMP + 0.2 mM calcium after a 2-min exposure to Mg-EGTA solution demonstrate that the inhibitory effect of calcium has been abolished. *(D, E, F)* Separate 3-min applications of NEM, SIN-1 and SNC do not induce channel activation even after ablation of the calcium binding factor. Each experiment was separated by a 2-min DTT wash.

In the presence of 200 μM intracellular calcium, this factor causes a large (~30-fold) reduction in the sensitivity of the channels to cyclic nucleotides and is readily ablated from the channels by a 2-min application of Mg-EGTA solution (Lynch & Lindemann, 1994; Balasubramanian et al., 1996). However, as shown in the example in Fig. 3, ablation of the calcium binding factor from the intracelluar membrane surface had no effect on the ability of either NEM, SIN-1 or SNC to activate the channel when they were applied for 3–5 min in each of 8 patches. It remains possible, however, that other unknown regulatory factors are involved in occluding the putative cysteine activation site(s) of the rat CNG channel.

Inhibition of CNG Channels By NO

An unexpected finding was that the cysteine modifying agents inhibited the cAMP-gated current. For example, a 2-min patch exposure to 100 μM SIN-1 caused an approximately 50% inhibition (Fig. 4*A*). In this experiment, SIN-1 was applied for 2 min in the absence of cAMP, and the inhibition was observed when cAMP was re-applied following removal of SIN-1. The inhibition displayed no voltage-dependence and did not spontaneously reverse for at least 5 min following SIN-1 removal ($n = 3$ patches). However, it was rapidly and completely reversed following a 2-min patch exposure to 2-mM DTT (Fig. 4*A*). Results averaged from 4 cells exposed to 100 μM SIN-1 for 2 min are displayed in Fig. 4*D*. A 2-min patch exposure to 50 μM SNC also produced an irreversible inhibition which was also completely reversed by a 2-min exposure to DTT (Fig. 4*B*). Averaged results from 4 cells exposed to 50 μM SNC are shown in Fig. 4*D*. Similarly, a higher (1mM) concentration of SNC induced a stronger inhibition which was almost completely reversed by a 2-min DTT exposure ($n = 7$ patches; Fig. 4*D*). In addition, 200 μM DTNB ($n = 7$ patches) also caused a strong irreversible inhibition (Fig. 4*C* and *D*). However, 100 μM NEM had no significant inhibitory effect when applied for 3 min in each of 4 patches (Fig. 4*D*). As discussed in detail below, since the NO-incuduced inhibition is mimicked by the oxidizing agent DTNB and reversed by DTT, it is highly likely that the inhibition is caused by NO^+-mediated oxidation of closely apposed cysteine residues.

The state dependence of NO inhibition was investigated by examining whether co-application of NO with a saturating concentration of cAMP had a different effect to that elicited by NO when applied alone. As seen in the example in Fig. 5*A*, when 1 mM SNC was co-applied with 100 μM cAMP for 2 min, no inhibition was observed. However, when the same SNC concentration was applied prior to cAMP in the same patch, a strong inhibition resulted. This inhibition was completely reversed by a 2-min application of DTT (*not shown*). A summary of results pooled from 4 patches exposed to 1 mM SNC is shown in Fig. 5*C*. The state-dependence of the DTNB-induced inhibition was also examined using a similar protocol. As seen in the example in Fig. 5*B*, 200 μM DTNB + 100 μM cAMP caused no inhibition when applied for 2 min, whereas in the same patch, 200 μM DTNB applied alone induced a strong inhibition. Results averaged from 4 cells are displayed in Fig. 5*C*. Thus, neither NO nor DTNB is effective in inhibiting the channel when it is in the open state.

A recent study (Gordon, Varnum & Zagotta, 1997) found that disulfide bond formation strongly potentiated currents in recombinantly expressed rod photoreceptor α homomeric CNG channels by reducing the concentration of cyclic nucleotide necessary for half-activation of the channels. This was investigated in the present study by examining the effects of 1 mM SNC and 200 μM DTNB at cAMP concentrations of 100, 2 and 0.5 μM. These cAMP concentrations were chosen as they represent saturating, half-saturating and barely threshold concentrations, respectively (Frings et al., 1992). As shown in

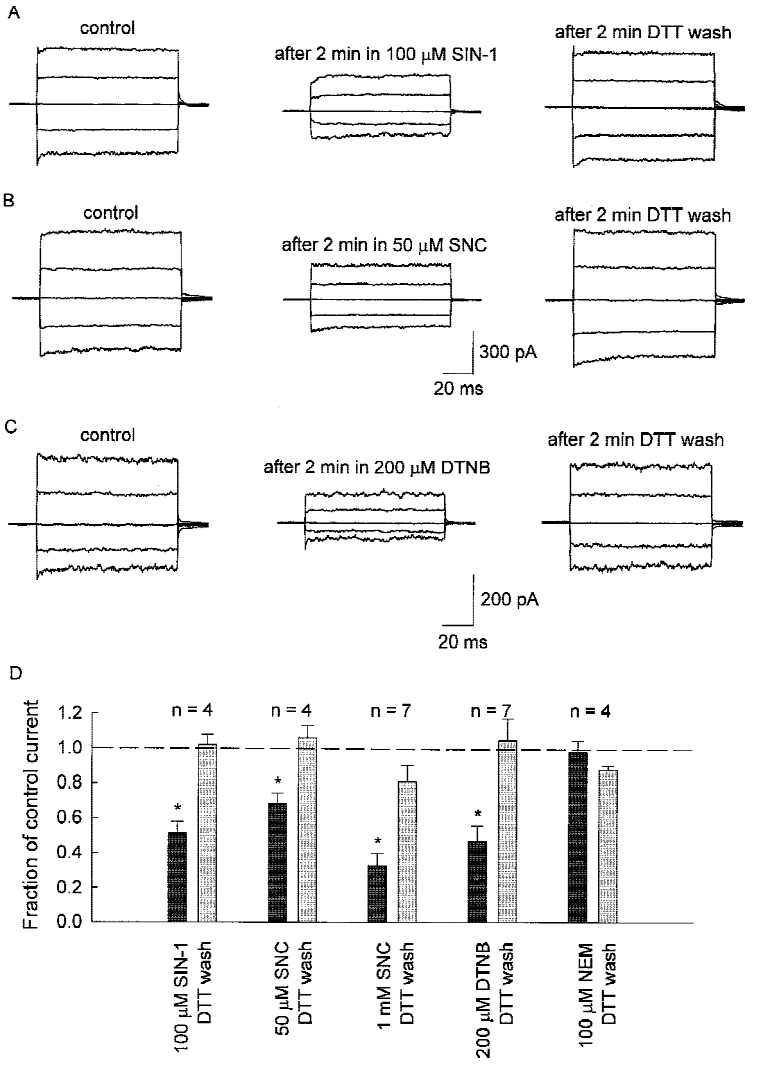

Fig. 4. Inhibition of the channels by sulfhydryl binding agents and its reversal by DTT. All displayed traces were recorded in response to a saturating (100 μM) concentration of cAMP. *(A)* Application of 100 μM cAMP revealed that this patch contained about 500 cAMP-activated channels (left panel). The patch was then exposed for 2 min to 100 μM SIN-1 in the absence of cAMP. When cAMP was reapplied, the current had significantly diminished (center panel). Then, following a 2-min patch exposure to 2-mM DTT, the cAMP-gated current had completely returned to control level (right panel). *(B)* Same patch as *A*. Example of a similar experiment to *A* except that 50 μM SNC was used in place of SIN-1. Scale bars in *B* apply also to *A*. *(C)* Similar experiment to *A* except that 200 μM DTNB was used in place of SIN-1. This patch contained about 278 active channels. *(D)* Averaged current responses to 2-min exposures to the indicated sulfhydryl-binding agents. All values are expressed as fractions of the original (control) cAMP-gated current. The numbers of experimental determinations (*n* values) are indicated for each experiment. Asterisks indicate values which were significantly different to the initial control current using a paired *t*-test and a *P* value of < 0.025.

the example in Fig. 6*A*, a 2-min application of 200 μM DTNB induced a similar degree of inhibition at all cAMP concentrations, but did not appear to change the sensitivity of the channels to cAMP. Indeed, results pooled from 3 patches clearly demonstrate that DTNB does not cause any significant change in the cAMP sensitivity of these channels (Fig. 6*C*, upper panel). As shown in the example in Fig. 6*B*, a similar situation was observed following a 2-min patch exposure to 1 mM SNC. Results averaged from 3 separate experiments, summarized in Fig. 6*C*, lower panel, demonstrate that SNC also does not induce any significant shift in the cAMP sensitivity of the channels.

Discussion

In contrast to recent reports on a variety of CNG channels, the present study demonstrates that the rat olfactory CNG channel is neither activated nor potentiated by cysteine modification. Instead, the channels are shown to be inhibited by the NO donors, SNC and SIN-1, and that this inhibition is reversed by DTT. In apparent contradiction to the present report, Broillet and Firestein (1997) reported that NO directly activated the native rat olfactory CNG channel. It is difficult to reconcile these results as Broillet and Firestein did not state the length of time NO compounds were applied, nor how many active CNG channels were present in their patches. However, it remains possible that the difference in results may reflect differences in NO application time. In the present study, NO was applied for periods of up to 10 min, which is at least double the amount of time required for the effects of NO donors to reach steady-state in other channels, including the ryanodine receptor (Xu et al., 1998), the NMDA receptor (Lei et al., 1992), gap junctional channels (Lu & McMahon, 1997), L-type calcium channels (Hu et al., 1997) and calcium-activated potassium

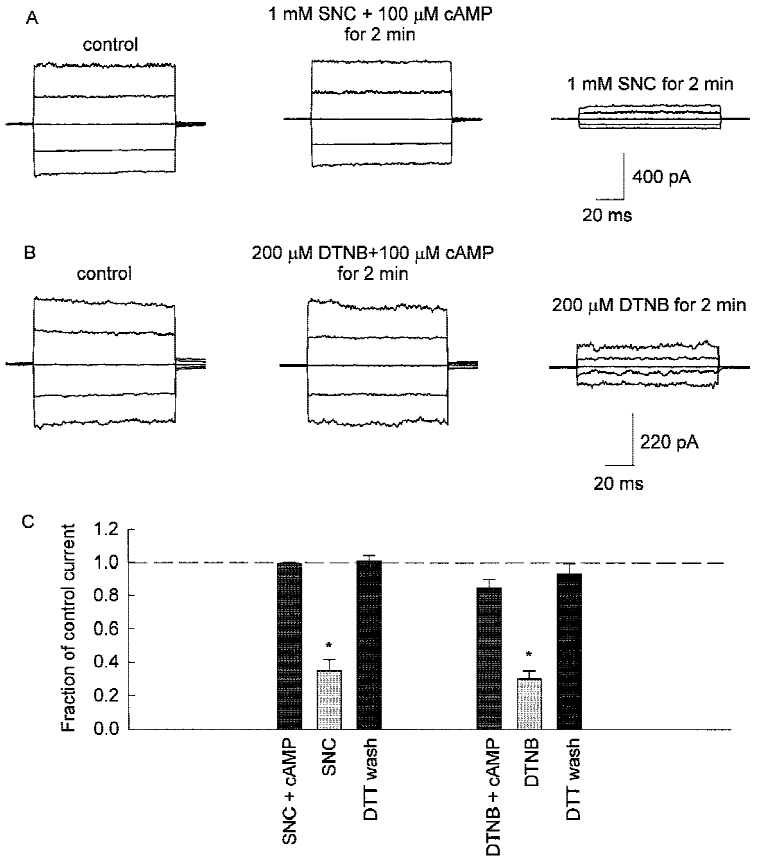

Fig. 5. State-dependence of the inhibition induced by NO and DTNB. All currents were recorded in response to a saturating (100 μM) concentration of cAMP. *(A)* Application of 100 μM cAMP indicated a total of about 550 active channels (left panel). The patch was then exposed to 1 mM SNC + 100 μM cAMP for 2 min and the cAMP-gated current was measured (center panel). Following this, the patch was exposed to 1 mM SNC alone for 2 min. Then the SNC was removed and cAMP was reapplied, revealing a significant degree of inhibition (right panel). A 2-min application of DTT subsequently returned the cAMP-gated current to the control level (not shown). *(B)* Example of a similar experiment to *A* except that 200 μM DTNB was used in place of 1 mM SNC. This patch contained a total of approximately 302 active channels. *(C)* Averaged responses from 4 patches exposed to 1 mM SNC and 4 patches exposed to 200 μM DTNB. The currents recorded in each patch following a 2-min DTT wash are also shown. Asterisks indicate significant differences to control levels as determined by a paired *t*-test and a *P* value of < 0.025.

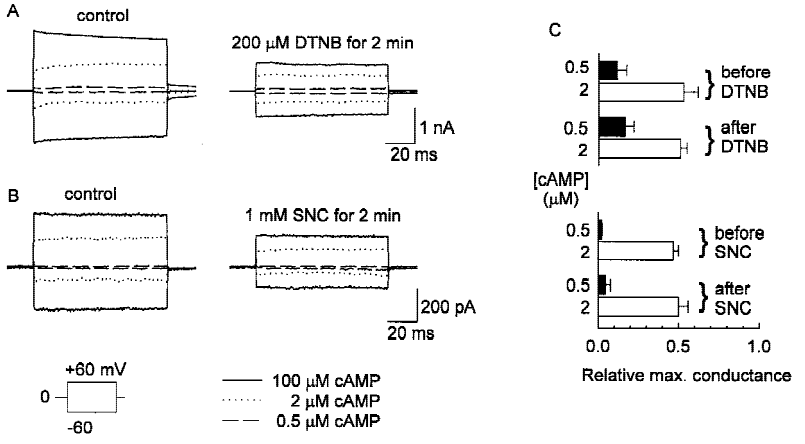

Fig. 6. Neither DTNB nor NO modify the apparent affinity of the channels for cAMP. All currents were recorded in response to a saturating (100 μM) concentration of cAMP and only currents in response to voltages steps from 0 mV to −60 and +60 mV are shown. *(A)* Currents activated by 100, 2 and 0.5 μM cAMP in one patch are shown (left panel). This patch contained a total of about 1,760 active channels. A 2-min application of 200 μM DTNB induced an overall inhibition, but no apparent change in the sensitivity of the channels to 2 or 0.5 μM cAMP (right panel). *(B)* Similar experiment to *A* except that 1 mM SNC was used instead of 200 μM DTNB. This patch contained about 320 active channels. *(C)* Relative conductances activated by 2 and 0.5 μM cAMP both before and after 2-min exposures to 200 μM DTNB (upper panel) and 1 mM SNC (lower panel). All displayed values are the averages of 3 experimental determinations. Using a paired *t*-test, with a *P* value of 0.025, neither DTNB nor SNC induced any significant change in the relaitve magnitudes of currents activated by 2 or 0.5 μM cAMP.

channels (Cai & Sauve, 1997). Even if olfactory CNG channel activation requires an NO application time greatly exceeding 10 min, it might be expected that at least a small fraction of the several hundred channels present in our patches may have been activated within 10 min. Thus, if NO activates the native rat olfactory CNG channel at all, it must act in an exceptionally slow and weak manner.

Although NO can undergo a range of chemical reactions under physiological conditions, its action on proteins is mainly due to NO^+-mediated oxidation of cysteine thiol groups (R-SH) to yield S-nitrosothiols (R-SNO)

(reviewed in Ignarro, 1990*a* and b). Although S-nitrosothiol groups are short-lived, a relatively persistent protein modification can result if two neighboring S-nitrosothiols combine to form a disulfide bond (R-S-S-R)(Ignarro, 1990*b*). Such a chemical modification is highly likely to have caused the persistent inhibition seen in this study. This interpretation is strongly supported by the dual observations that the oxidizing agent, DTNB, had an identical effect to NO, and that the disulfide bond reducing agent, DTT, reversed the inhibition by both substances. It is important to note that DTT had no effect on channels which had not previously been exposed to exogenous cysteine-binding agents. The absence of significant NEM-induced inhibition indicates that the alkylation of one or both of the paired cysteines does not replicate the effect of cysteine crosslinking. Since the reactive cysteines are capable of forming disulfide bonds only in the closed channel state (Fig. 5), it may be concluded that the structural rearrangements accompanying channel activation either separate the cysteines or occlude at least one of them.

NO has previously been reported to indirectly activate CNG channels in several experimental systems. Lishka and Schild (1993) found that NO activated a cation conductance in *Xenopus laevis* olfactory receptor neurons provided that guanosine triphosphate was present in the intracellular solution. This finding suggested that NO stimulated particulate guanylate cyclase to produce cGMP which in turn directly activated the channels. Similar mechanisms were postulated to explain NO activation of lizard cone photoreceptor CNG channels (Savchenko, Barnes & Kramer, 1997) and CNG channels in rat retinal ganglion cells (Ahmad et al., 1994).

Direct effects of NO and other cysteine modifying agents on CNG channels have also been examined. Studies on the photoreceptor CNG channel revealed that cysteine alkylation by NEM potentiates the cGMP-gated current by causing a decrease in the cGMP concentration required for half-maximal activation and a reduction in the cooperativity for cGMP binding (Balakrishnan, Padgett & Cone, 1990; Donner et al., 1990; Serre, Ildefonse & Bennett, 1995; Gordon et al., 1997). Moreover, several cysteine binding agents, including each of those used in the present study, were reported to act as agonists of the salamander olfactory CNG channel (Broillet & Firestein, 1996*a*). NO was also found to act as an agonist of the recombinantly expressed rat olfactory CNG α homomeric channel, the β homomeric channel and the rat α/β heteromeric channel (Broillet & Firestein, 1997). Together, these results demonstrate that the effects of cysteine modification vary from one CNG channel isoform to another. This is not surprising since the number and location of cysteines also varies between CNG channel isoforms. However, it is important to note that none of these studies reported any inhibitory effect of sulfhydryl binding agents even when used at concentrations of up to 20-fold higher than those used in the present study. Thus, NO-induced inhibition appears to be a unique property of the native rat olfactory CNG channel.

Thus, native rat olfactory CNG channels differ from recombinantly expressed rat α or β homomers or α/β heteromers in that they are not activated by NO, and that they are inhibited by NO. Since methodological differences cannot readily explain these differences, the most plausible explanation is that the native rat CNG channel has a different structural arrangement of exposed cysteines to the α/β heteromer and that these cysteines are functionally coupled to the channel in a different manner. Such a structural difference would presumably be conferred by an as yet unidentified subunit or tightly bound regulatory factor. Such a factor must exert a dominant influence on the channel gating process if it not only prevents the channels from being activated by cysteine modification, but also introduces a potent inhibitory effect.

The results presented here may also have an important physiological relevance. NO synthetase has been localized to vertebrate olfactory sensory neurons, implying a physiological role for NO (Roskams et., 1994; Bredt & Snyder, 1994; Zhao, Firestein & Greer, 1994). Indeed, possible effector mechanisms for NO and their physiological consequences for olfactory transduction have recently been discussed (Breer & Shepherd, 1993; Broillet & Firestein, 1996*b*; Zufall, Shepherd & Barnstable, 1997). Although it has not been established that the inhibitory effect described here occurs in olfactory receptor neurons in vivo, such a mechanism is feasible since S-nitrosylation-induced sulfhydryl crosslinking has previously been shown to inhibit NMDA-activated channels in intact central neurons (Lei et al., 1992). Since the olfactory CNG channel is highly permeant to calcium (Frings et al., 1995), the NO-induced inhibition described here is potentially an important physiological modulator of many intracellular processess in mammalian olfactory receptor neurons.

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References

Ahmad, I., Leinders-Zufall, T., Kocsis, J.D., Shepherd, G.M., Zufall, F., Barnstable, C.J. 1994 Retinal ganglion cells express a cGMP-gated cation conductance activatable by nitric oxide donors. *Neuron* **12:**155–165

Balakrishnan, K., Padgett, J., Cone, R.A. 1990. Calcium influx in rod outer segment patches: NEM potentiates the effects of cGMP. *Biophys. J.* **57:**371*a* (*Abstr.*)

Balasubramanian, S., Lynch, J.W., Barry, P.H. 1996 Calcium-dependent modulation of the agonist affinity of the mammalian

olfactory cyclic nucleotide-gated channel by calmodulin and a novel endogenous factor. *J. Membrane Biol.* **152:**13–23

Bradley, J., Li, J., Davidson, N., Lester, H.A., Zinn, K. 1994. Heteromeric olfactory cyclic nucleotide-gated channels: a subunit that confers increased sensitivity to cAMP. *Proc. Nat. Acad. Sci. USA* **91:**8890–8894

Bredt, D.S., Snyder, S.H. 1994. Transient nitric oxide synthase neurons in embryonic cerebral cortical plate, sensory ganglia, and olfactory epithelium. *Neuron* **13:**301–313

Breer, H., Shepherd, G.M. 1993. Implications of the NO/cGMP system for olfaction. *Trends Neurosci.* **16:**5–9

Broillet, M.C., Firestein, S. 1996*a*. Direct activation of the olfactory cyclic nucleotide-gated channel through modification of sulfhydryl groups by NO compounds. *Neuron* **16:**377–381

Broillet, M.C., Firestein, S. 1996*b*. Gaseous second messengers in vertebrate olfaction. *J. Neurobiology* **30:**49–57

Broillet, M.C., Firestein, S. 1997. β subunits of the olfactory cyclic nucleotide-gated channel form a nitric oxide activated Ca^{2+} channel. *Neuron* **18:**951–958

Cai, S., Sauve, R. 1997. Effects of thiol-modifying agents on a K(Ca^{2+}) channel of intermediate conductance in bovine aortic endothelial cells. *J. Membrane Biol.* **158:**147–158

Chiu, P., Lynch, J.W., Barry, P.H. 1997. Odorant-induced currents in intact patches from rat olfactory receptor neurons: theory and experiment. *Biophys. J.* **72:**1442–1457

Dhallon, R.S., Yau, K.-W., Schrader, K.A., Reed, R.R. 1990. Primary structure and functional expression of a cyclic nucleotide-activated channel from olfactory neurons. *Nature* **347:**184–187

Donner, K., Hemila, S., Kalamkarov, G., Koskelainen, A., Pogozheva, I., Rebrik, T. 1990. Sulfhydryl binding reagents increase the conductivity of the light-sensitive channel and inhibit phototransduction in retinal rods. *Exp. Eye Res.* **51:**97–105

Finn, J.T., Grunwald, M.E., Yau, K.-W. 1996. Cyclic nucleotide-gated ion channels: an extended family with diverse functions. *Annu. Rev. Physiol.* **58:**395–426

Finn, J.T., Li, J., Yau, K.-W. 1995. C-terminus involvement in the gating of cyclic nucleotide-activated channels as revealed by Ni^{2+} and NEM. *Biophys. J.* **68:***A*253 (*Abstr.*)

Frings, S., Lynch, J.W., Lindemann, B. 1992. Properties of cyclic nucleotide-gated channels mediating olfactory transduction: activation, selectivity and blockage. *J. Gen. Physiol.* **100:**45–67

Frings, S., Seifert, R., Godde, M., Kaupp, U.B. 1995 Profoundly different calcium permeation and blockage determine the specific function of distinct cyclic nucleotide-gated channels. *Neuron* **15:**169–179

Gordon, S.E., Varnum, M.D., Zagotta, W.N. 1997. Direct interaction between amino- and carboxyl-terminal domains of cyclic nucleotide-gated channels. *Neuron* **19:**431–441

Hu, H., Chiamvimonvat, N., Yamagishi, T., Marban, E. 1997. Direct inhibition of expressed cardiac L-type Ca2+ channels by S-nitrosothiol nitric oxide donors. *Circ. Res.* **81:**742–752

Ignarro, L.J. 1990*a*. Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annu. Rev. Pharmacol. Toxicol.* **30:**535–560

Ignarro, L.J. 1990*b*. Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. *Circ. Res.* **65:**1–21

Jan, L.Y., Jan, Y.N. 1990. A superfamily of ion channels. *Cell* **69:**715–718

Kaupp, U.B. 1995. Family of cyclic nucleotide gated ion channels. *Curr. Opin. Neurobiol.* **5:**434–442

Kaupp, U.B., Niidome, T., Tanabe, T., Terada, S., Bönigk, W., Stühmer, W., Cook, N.J., Kangawa, K., Matsuo, H., Hirose, T., Miyata, T., Numa, S. 1989. Primary structure and functional expression from complementary DNA of the rod photoreceptor cyclic GMP-gated channel. *Nature* **342:**762–766

Lei, S.Z., Pan, Z.H., Aggarwal, S.K., Chen, H.S.V., Hartmann, J., Sucher, N.J., Lipton, S.A. 1992. Effect of nitric oxide modulation on the redox modulatory site of the NMDA receptor-channel complex. *Neuron* **8:**1087–1099

Liman, E.R., Buck, L.B. 1994. A second subunit of the olfactory cyclic nucleotide-gated channel confers high sensitivity to cAMP, *Neuron* **13:**611–621

Lischka, F.W., Schild, D. 1993. Effects of nitric oxide upon olfactory receptor neurons in Xenopus laevis. *Neuroreport* **4:**582–584

Liu, D.T., Tibbs, G.R., Siegelbaum, S.A. 1996. Subunit stoichiometry of cyclic nucleotide-gated channels and effects of subunit order on channel function. *Neuron* **16:**983–990

Lu, C., McMahon, D.G. 1997. Modulation of hybrid bass gap junctional channel gating by nitric oxide. *J. Phsyiol.* **499:**689–699

Lynch, J.W., Barry, P.H. 1991. Properties of transient K^+ currents and underlying single K^+ channels in rat olfactory receptor neurons. *J. Gen. Physiol.* **97:**1043–1072

Lynch, J.W., Lindemann, B. 1994. Cyclic nucleotide-gated channels of rat olfactory receptor cells: divalent cations control the sensitivity to cAMP. *J. Gen. Physiol.* **103:**87–106

Roskams, A.J., Bredt, D.S., Dawson, T.M., Ronnett, G.V. 1994. Nitric oxide mediates the formation of synaptic connections in developing and regenerating olfactory receptor neurons. *Neuron* **13:**289–299

Savchenko, A., Barnes, S., Kramer, R.H. 1997. Cyclic-nucleotide-gated channels mediate synaptic feedback by nitric oxide. *Nature* **390:**694–698

Serre, V., Ildefonse, M., Bennett, N. 1995. Effects of cysteine modification on the activity of the cGMP-gated channel from retinal rods. *J. Membrane Biol.* **146:**145–162

Xu, L., Eu, J.P., Meissner, G., Stamler, J.S. 1998. Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science* **279:**234–237

Zhao, H., Firestein, S., Greer, C.A. 1994. NADPH-diaphorase localization in the olfactory system. *Neuroreport* **6:**149–152

Zufall, F., Shepherd, G.M., Barnstable, C.J. 1997. Cyclic nucleotide gated channels as regulators of CNS development and plasticity. *Curr. Opin. Neurobiol.* **7:**404–412