A Novel Pharmacological Probe Links the Amiloride-Insensitive NaCl, KCl, and NH₄Cl Chorda Tympani Taste Responses

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Received 25 May 2001; accepted in final form 31 July 2001

DeSimone, John A., Vijay Lyall, Gerard L. Heck, Tam-Hao T. Phan, Rammy I. Alam, George M. Feldman, and R. Michael Buch. A novel pharmacological probe links the amiloride-insensitive NaCl, KCl, and NH₄Cl chorda tympani taste responses. J Neurophysiol 86: 2638–2641, 2001. Chorda tympani taste nerve responses to NaCl can be dissected pharmacologically into amiloride-sensitive and -insensitive components. It is now established that the amiloride-sensitive, epithelial sodium channel acts as a sodium-specific ion detector in taste receptor cells (TRCs). Much less is known regarding the cellular origin of the amiloride-insensitive component, but its anion dependence indicates an important role for paracellular shunts in the determination of its magnitude. However, this has not precluded the possibility that undetected apical membrane ion pathways in TRCs may also contribute to its origin. Progress toward making such a determination has suffered from lack of a pharmacological probe for an apical amiloride-insensitive taste pathway. We present data here showing that, depending on the concentration used, cetylpyridinium chloride (CPC) can either enhance or inhibit the amiloride-insensitive CT response to NaCl. The CPC concentration giving maximal enhancement was 250 μM. At 2 mM, CPC inhibited the entire amiloride-insensitive part of the NaCl response. The NaCl response is, therefore, composed entirely of amiloride- and CPC-sensitive components. The magnitude of the maximally enhanced CPC-sensitive component varied with the NaCl concentration and was half-maximal at [NaCl] = 62 ± 11 (SE) mM. This was significantly less than the corresponding parameter for the amiloride-sensitive component (268 ± 71 mM). CPC had similar effects on KCl and NH₄Cl responses except that in these cases, after inhibition with 2 mM CPC, a significant CPC-insensitive response remained. CPC (2 mM) inhibited intracellular acidification of TRCs due to apically presented NH₄Cl, suggesting that CPC acts on an apical membrane nonselective cation pathway.

INTRODUCTION

Amiloride inhibits part of the chorda tympani (CT) response to NaCl, an observation that led to the identification of the epithelial sodium channel (ENaC) as a transducer in NaCl taste (Stewart et al. 1997). CT recordings to Na salts also show an amiloride-insensitive (AI) response that is anion dependent (Elliott and Simon 1990; Formaker and Hill 1988). It was proposed that anions exert their influence through modulation of transepithelial potentials set up across paracellular shunts in the taste buds (Elliott and Simon 1990), and this was proved by obtaining the CT responses to Na salts under tissue voltage clamp (Ye et al. 1994).

CT nerve recordings

Sprague-Dawley rats (150–200 g) were prepared for recording as previously described (Ye et al. 1994). Neural responses were ampli-
fied, filtered, full-wave rectified, and integrated with a time constant of 1 s. The rinse solution was 10 mM KCl. Typically stimulus solutions remained on the tongue for 2 min. Control stimuli consisting of 300 mM NaCl and 300 mM NH₄Cl, applied at the beginning and at the end of an experiment, were used to assess preparation stability. Stimuli consisted of NaCl solutions ranging from 20 to 500 mM, 300 mM KCl, and 100 mM NH₄Cl. The cetylpyridinium chloride (CPC, Sigma, St. Louis, MO) dose versus CT response relation was obtained using responses to 100 mM NaCl as baseline. CPC concentrations were (in mM): 50, 100, 250, 500, 1,000, and 2,000. The data were digitized and analyzed off-line. Responses were taken as the area under the response curve over the first minute of stimulation. The displayed CT responses in Figs. 1 and 2 are representative results from at least four separate experiments.

Intracellular pH measurement

To monitor the flux of NH₄⁺ ions across the apical membranes of polarized TRCs we used a separate in vitro preparation of a single fungiform taste bud (Lyall et al. 2001). Changes in intracellular pH (pHᵢ) were measured by imaging TRCs using the fluoroprobe, BCECF (Lyall et al. 2001). A decrease in pHᵢ indirectly indicates the apical entry of NH₄⁺ ions into TRCs. The TRCs were perfused on both sides with control solution containing (in mM) 150 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES, pH 7.4. The temporal changes in pHᵢ were monitored following the exposure of the apical membrane to a similar solution containing 150 mM NH₄Cl at pH 7.4 in the presence and absence of 2 mM CPC.

RESULTS

Figure 1C shows that CPC caused the NaCl response to increase between 50 and 250 μM. Beyond 250 μM CPC, NaCl responses decreased reaching control level at ~700 μM. At 1 and 2 mM CPC, NaCl responses were less than control values. Figure 1A shows the effect of 250 μM CPC on the response to 0.3 M NaCl. When NaCl was displaced by NaCl + 250 μM CPC, the response increased rapidly to a higher level until rinsed from the tongue. Following a second NaCl stimulation, the AS response was eliminated in the presence of 100 μM amiloride. Adding 250 μM CPC in the presence of amiloride gave the same magnitude enhancement observed without amiloride, indicating that the AS and CPC-sensitive pathways are independent. Figure 1B shows the effect of 2 mM CPC on the response to 0.3 M NaCl. The presence of 2 mM CPC suppressed the response by 20%. Amiloride suppressed a second NaCl stimulation by 80%. Addition of 2 mM CPC reduced the response to baseline levels indistinguishable from the rinse.

FIG. 1. A: the effect of 250 μM cetylpyridinium chloride (CPC) on the integrated chorda tympani response to 300 mM NaCl and 300 mM NaCl + 100 μM amiloride. CPC enhanced the response by the same magnitude in each case. B: the effect of 2 mM CPC on the integrated chorda tympani response to 300 mM NaCl and 300 mM NaCl + 100 μM amiloride. CPC suppressed the entire amiloride-insensitive (AI) part of the response. C: the effect of increasing CPC concentration on the response to 100 mM NaCl. Rᵣₚᵣ is the response to 100 mM NaCl containing a given concentration of CPC. Rᵣ₀.₁ M NaCl is the response to 100 mM NaCl. Rᵣₚᵣ/Rᵣ₀.₁ M NaCl > 1 indicates an enhanced response, Rᵣₚᵣ/Rᵣ₀.₁ M NaCl < 1 indicates a suppressed response. Values represent the means ± SE (n = 4)
response level, i.e., the NaCl response is composed entirely of AS and CPC-sensitive components.

The response to 300 mM KCl was increased by 50% in the presence of 250 mM CPC and decreased by 35% by 2 mM CPC (Fig. 2A). The response to 100 mM NH4Cl was increased by 40% in the presence of 250 mM CPC and decreased by 30% by 2 mM CPC (Fig. 2B). In each case, a significant part of the response was CPC insensitive.

The influx of NH4+ ions into TRCs from the apical side was observed in polarized TRCs. When the TRCs were bathed symmetrically in control solution, the mean pH_i was 7.48 (cf. Fig. 2C). The pH response to NH4Cl depends on the relative permeability of NH4+ and its conjugate base NH3. Replacement of the apical control solution by the isosmotic 150 mM NH4Cl caused pH_i to decrease, indicating that the acidic form, NH4+, can enter TRCs faster from the apical side than the base NH3. Inside the TRCs NH4+ gives up H+ and forms NH3, which escapes. In contrast, when 150 mM NH4Cl + 2 mM CPC was then placed on the apical side, pH_i increased rapidly indicating a decrease in the apical NH4+ permeability relative to NH3.

The CPC-enhanced NaCl response was studied over a range of NaCl concentrations (cf. Fig. 3). NaCl + 250 μM CPC was a saturating function of NaCl concentration with K_m = 185 ± 35 mM. The CPC-sensitive component, obtained with NaCl + 100 μM amiloride + 250 μM CPC, had K_m = 62 ± 11 mM. The AS component had K_m = 268 ± 71 mM.

**DISCUSSION**

Single units in the CT that respond nonselectively to various cations may imply TRCs that use nonselective cation channels as transducers. Investigation of this hypothesis has been im-
peded, however, by the lack of an effective pharmacological probe for such TRCs. CPC acts on the AI part of the CT response to NaCl, where it either reversibly enhances or suppresses the response. At the suppressing concentration of 2 mM, the blocking effect of CPC is additive with that of amiloride, indicating that NaCl responses are composed of two pharmacologically independent inputs. CPC has essentially similar effects on responses to KCl and NH₄Cl. In the latter case, CPC was shown to reduce the apical influx of NH₄⁺ into TRCs. This along with the rapidity and reversibility of CPC action suggest that the CPC-sensitive pathway is probably an apical membrane nonselective cation conductance. For KCl and NH₄Cl, there remain significant CPC-insensitive transduction pathways. We note that both pharmacological actions of CPC occur at concentrations above the CPC critical micelle concentration (Simoncîc and Špan 1998), so the CPC actions reported here cannot be attributed to monomer-micelle transformations occurring within that concentration range.

A comparison of the parameters of the AS and CPC-sensitive parts of the NaCl response in Fig. 3 show the former to be a high-capacity, low-affinity system and the latter to be a low-capacity, high-affinity system similar respectively to N- and H-fiber types. Estimates of $K_m$ values for N and H fibers are 220 and 81 mM, respectively (Frank et al. 1983), which compare well with 268 ± 71 and 62 ± 11 mM found here, respectively, for the AS and CPC-sensitive parts of the NaCl response. The CPC-sensitive component, like the H-fiber response, has an impact on the low concentration NaCl response and therefore has a role in determining thresholds.

This work was supported by National Institute on Deafness and Other Communication Disorders Grant DC-02422 and by a grant from GlaxoSmithKline.

REFERENCES


J Neurophysiol • VOL 86 • NOVEMBER 2001 • www.jn.org