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Evidence for Two Separate Purinergic Responses in *Paramecium tetraurelia*: XTP Inhibits Only the Oscillatory Responses to GTP

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Abstract. The purine nucleotide GTP causes a complex behavioral response and two distinct electrophysiological responses in the ciliated protozoan *Paramecium tetraurelia.* One of the two electrophysiological responses is an oscillating current that is responsible for the repeated backward swimming episodes that constitute the behavioral response to GTP. The second electrophysiological response is a sustained current whose relationship to the first is unknown. Here we show that the purine nucleotide XTP can completely block both the behavioral response to GTP and its associated oscillating current, but not the sustained current induced by GTP. Notably, XTP alone causes a sustained current similar to that induced by GTP. We believe the data support the notion that *P. tetraurelia* possesses two distinct signal transduction pathways sensitive to purine nucleotides: one specific for GTP that leads to oscillating currents and behavior, and a second pathway activated by GTP and other purine nucleotides that leads to a sustained current.

Key words: Purinoceptor — ATP — Calcium oscillations — Receptor antagonist — Purine nucleotide — Behavior — Ciliate

Introduction

Paramecium tetraurelia responds to the purine nucleotide GTP with an unusual display of oscillatory swimming behavior (Clark, Hennessey & Nelson, 1993). The cell normally propels itself forward through its freshwater medium by the power of the thousands of cilia covering its body. Stimuli such as touch, heat, gravity, ions and organic chemicals cause cells either to swim faster forward (e.g., if the stimulus is an attractant or a touch to the posterior) or to jerk backward briefly (e.g., if the stimulus is a repellent or a touch to the anterior) (Eckert, 1972; Machemer, 1976; Machemer, 1988; Preston & Saimi, 1990; Schultz, Klumpp & Hinrichsen, 1990; Bonini et al., 1991; Hinrichsen, 1993). Unlike these other stimuli, extracellular GTP triggers repeated and long (~2.5 sec) backward swimming episodes that are often punctuated with periods of brief whirling, in which a cell gyrates about its lateral axis. This stereotypical ''GTP response'' can last as long as 10 min or more, although whirling behavior is more prevalent earlier in the response than later. While the response can be triggered by concentrations of GTP as low as 100 nM, higher concentrations cause increased backward swimming, with 10 μM GTP eliciting a maximal response (Clark et al., 1993). *P. tetraurelia* responds preferentially to GTP and its close structural analogues, such as GTP-γ-S and GMP-PNP. ATP is 1,000-fold less potent, while other nucleotides such as CTP, XTP, UTP, and ITP produce no backward swimming (Clark et al., 1993). The specificity of the response suggests that it may be mediated by a receptor, although to date no receptor has been identified.

The swimming behavior of *Paramecium* is tightly coupled to the electrophysiological state of the membrane (Eckert, 1972; Kung & Saimi, 1982; Machemer, 1988; Preston & Saimi, 1990). Hyperpolarization leads to faster forward swimming, while depolarization causes backward swimming. Extracellular GTP causes backward swimming by triggering currents that depolarize the cell (Clark et al., 1997). Two types of currents are induced by GTP. The first is an oscillating current of about 750 pA that is caused by the concurrent influx of both Mg^{2+} and Na^{+}. (The amplitudes reported here were measured in cells bathed in testing solution, which contains both Mg^{2+} and Na^{+}. For precise ionic definition *see* Materials and Methods.) This current activates about 6 times per min and directly corresponds with episodes of

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backward swimming in the free-swimming cell. Eliminating the oscillating current, for example, by mutation (*GTP-insensitive A*) (Mimikakis, Nelson & Preston, 1998) or by treatment of the wild type with a membrane-permeant derivative of cAMP, N^6-benzoyladenosine-3′,5′ cyclic monophosphate (J.L. Mimikakis, K.D. Clark & D.L. Nelson, *in preparation*), completely abolishes the repetitive backward swimming response to GTP. Such cells do respond to GTP by whirling, however, suggesting that they are somehow still sensitive to this nucleotide.

The second current induced by GTP is a sustained current of 100–200 pA that decays slowly over 3–5 min but has not been ionically characterized. Neither the *GTP-insensitive* mutation nor treatment with cAMP derivatives measurably affects this current, suggesting that it may be responsible for whirling. Nonetheless, the relationship between the sustained current and the oscillating response to GTP remains unclear. For example, it is possible that the sustained current acts early in the GTP-transduction pathway to induce the oscillating current. In this scenario, *GTP-insensitive* and cAMP derivatives would block the pathway between the two currents, leaving the sustained current intact, but eliminating the oscillating one. Alternatively, the sustained current may be part of a separate pathway that can also be activated by GTP and is not affected by *GTP-insensitive* or cAMP derivatives. This paper examines the effects on behavior and electrophysiology of the purine nucleotide XTP, both in conjunction with GTP and alone. XTP blocks both the backward swimming and the oscillating current induced by GTP. In addition, we found that XTP alone causes a sustained current similar to that elicited by GTP. The data support the hypothesis that the oscillating current and the sustained current are part of two separate purinergic transduction pathways.

Materials and Methods

Cell Stocks and Culture Conditions

The present studies were conducted using *Paramecium tetraurelia*, stocks 51S (wild type) and *GTP-insensitive A* (*ginA*). Both the wild type and *ginA* stocks also contained the trichocyst nondischarge mutation *nd6* (Lefort-Tran et al., 1981), which facilitates insertion of microelectrodes during electrophysiological experiments, but does not otherwise affect behavioral or electrophysiological measurements. Cells were grown at room temperature (22–25°C) in wheat grass medium as described (Sonneborn, 1970).

Solutions

Several membrane ion conductances are known to be involved in the behavioral response of *P. tetraurelia* to GTP. ''Testing solution'' contained all the ions necessary for a strong behavioral response to GTP: 4 mM KCl, 1 mM Ca^{2+} ($CaCl_2$ and $Ca(OH)_2$), 1 mM HEPES buffer, 0.5 mM $MgCl_2$, and 5 mM NaCl, 10 μM EDTA, pH 7.2. This solution was used to test the swimming response to GTP and XTP. When testing the membrane potential response to these nucleotides, the solution was modified by the addition of 10 mM tetraethylammonium chloride (TEA).

Behavioral Assays

All cells were preincubated for at least 30 min in testing solution prior to testing. Individual cells were then selected with a micropipette and ejected forcibly into a testing solution containing GTP, ITP, or XTP as indicated in the text. Backward swimming episodes were recorded on a computer in real time, and expressed as the percentage of time a cell spent swimming backward during a 2-min assay period, as described in Clark et al. (1993).

Electrophysiological Assay

GTP-induced currents were measured using techniques as described (Clark et al., 1997). Capillary microelectrodes used to establish a voltage clamp contained 1 mM CsCl and had tip resistances of about 40 MΩ. Cell membranes were clamped at −15 mV. Cells were bathed in testing solution containing GTP and/or XTP as indicated in the text. The flow rate through the experimental chamber (capacity ~1 ml) was 10–15 ml/min. Currents were filtered at 10 Hz, and recorded on a chart recorder. All recordings were made at room temperature (22–25°C).

Abbreviations

GTP-γ-S, guanosine 5′-O-(3-thiotriphosphate); GMP-PNP, β,γ-imidoguanosine 5′-triphosphate

Results

XTP Blocks Behavior Induced by GTP

The purine nucleotide XTP completely blocked the oscillatory behavioral response elicited by GTP in wild-type *P. tetraurelia* (Fig. 1). Cells stimulated with 10 μM GTP alone swam backward repetitively for 20–25% of the time during a 2-min assay (Fig. 1*A*). XTP caused complete inhibition of the GTP response when both this nucleotide and GTP were present at 10 μM (Fig. 1*A*) ($n = 3$). Another purine nucleotide, ITP, also caused complete inhibition, but at higher concentrations (50 μM, $n = 8$) (*not shown*). XTP and ITP inhibited the GTP response in a dose-dependent manner; the concentration of XTP that inhibited the GTP response by 50% (IC_{50}) was 210 nM; the IC_{50} for ITP was 1.2 μM (Fig. 1*B*). While XTP alone did not cause the repetitive backward swimming behavior typical of cells in GTP, it did cause cells to whirl (*not shown*).

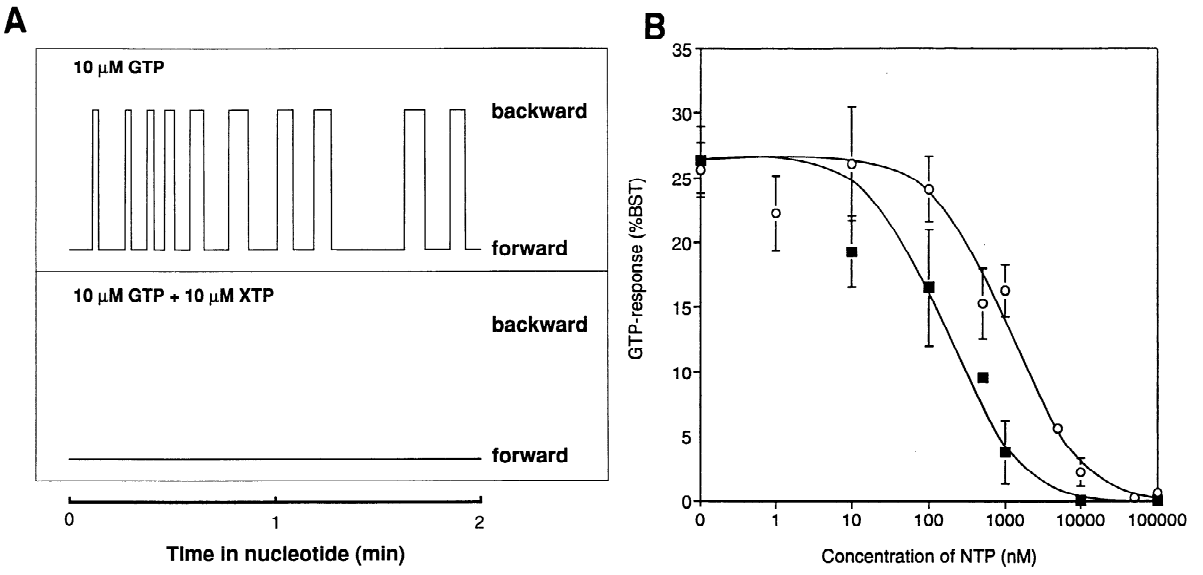

Fig. 1. Inhibition of the swimming response to GTP by XTP and ITP. (*A*) Backward and forward swimming patterns displayed by individual cells for 2 min. *Upper trace:* a single cell was transferred into 10 μM GTP at time = 0 as a control. *Lower trace:* a single cell was transferred at time = 0 into 10 μM GTP plus 10 μM XTP. (*B*) Dose-response curve showing inhibition of the repetitive backward swimming response to 10 μM GTP (expressed as %BST, backward swimming time) by increasing concentrations of XTP (■) or ITP (○). Each point represents the mean of 3 cells (XTP curve) or 4 to 20 cells (ITP curve). Error bars represent SEM.

XTP Blocks the Oscillating Current Induced by GTP

We compared the electrophysiological responses induced by GTP in the presence and absence of XTP. Both oscillating and sustained currents were elicited in voltage-clamped cells bathed in 10 μM GTP alone (Fig. 2, upper trace, $n = 3$). XTP (10 μM) blocked the oscillating current induced by 10 μM GTP, but not the sustained current when applied with GTP (Fig. 2, lower trace, $n = 6$). The sustained current was similar in amplitude to that produced by GTP alone (~175 pA). In no instance did these cells exhibit a current resembling the oscillating current produced by GTP alone.

XTP Induces a Sustained Current Similar to that Induced by GTP

We measured the currents produced by GTP and by XTP alone in both wild-type and *ginA* mutant cells. As above, GTP induced both an oscillating and a sustained current in the wild type (Fig. 3, upper left). The amplitude of the sustained current, as measured from the baseline to the base of the oscillating current, was 140 ± 11 pA ($n = 7$, mean ± SEM). When administered alone, 10 μM XTP induced a sustained current (Fig. 3, lower left) that was similar in form and amplitude (98 ± 29 pA; $n = 6$) to that induced by GTP. In no instance did XTP produce a current resembling the oscillating current produced by GTP. In *ginA* mutant cells, which responded to GTP

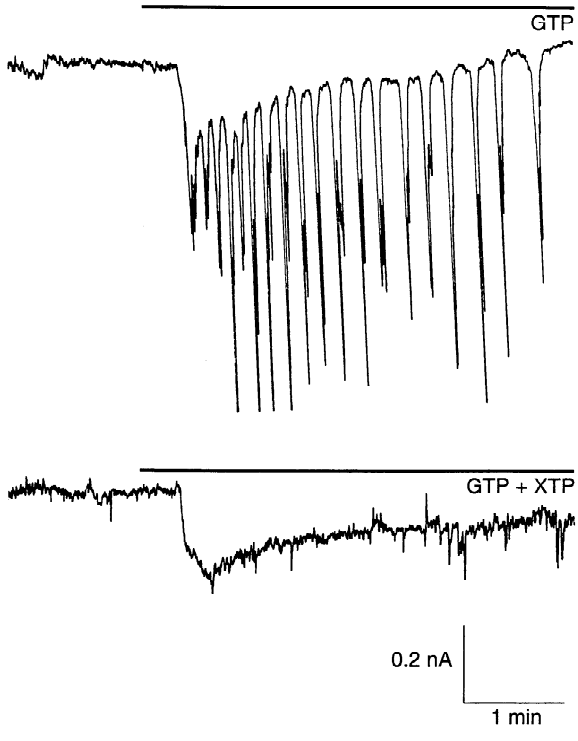

Fig. 2. Inhibition by XTP of the oscillating currents induced by GTP. *Upper trace:* Membrane currents recorded from a single cell placed under voltage clamp and bathed in 10 μM GTP (bar). Similar results were seen in 3 cells. *Lower trace:* Currents recorded from a cell under voltage clamp and bathed in 10 μM XTP together with 10 μM GTP (bar). Similar results were seen in 6 cells.

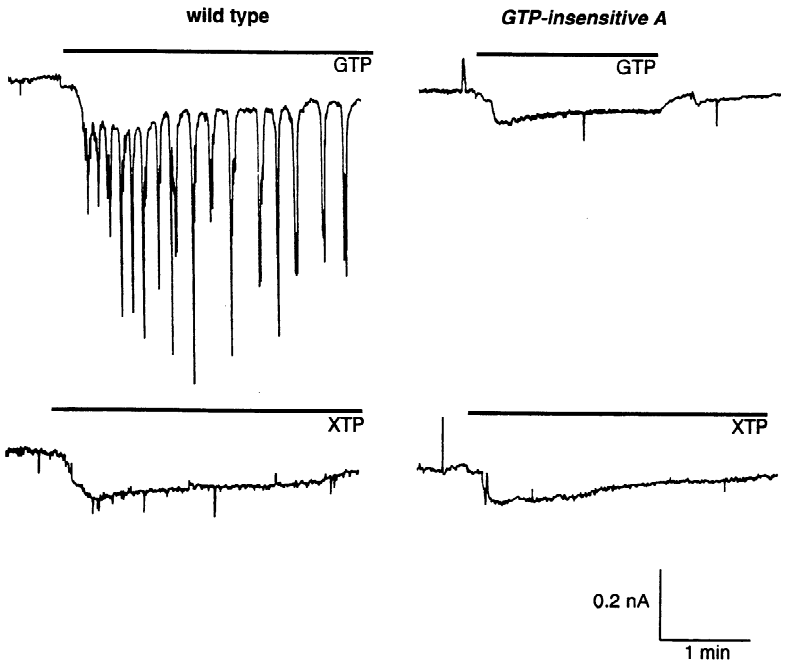

Fig. 3. Currents elicited by GTP or XTP from wild-type and *GTP-insensitive* mutant cells. *Left panel:* Membrane currents recorded from individual wild-type cells placed under voltage clamp and bathed in 10 μM GTP (*upper trace*) or 10 μM XTP (*lower trace*). *Right panel:* Currents recorded from individual *GTP-insensitive* mutant cells under voltage clamp and bathed in 10 μM GTP (*upper trace*) or 10 μM XTP (*lower trace*).

with only a sustained current (Fig. 3, upper right, $n = 3$), XTP elicited a sustained current resembling that produced by GTP alone (Fig. 3, lower right, $n = 3$).

Discussion

The purine nucleotide XTP completely blocks both the repeated backward swimming response and the oscillating current elicited by extracellular GTP. These results, combined with the specificity of the GTP response for guanosine nucleoside triphosphates, imply the existence of a receptor that can bind both GTP and XTP, but can be activated only by GTP. XTP is structurally similar to GTP, suggesting that XTP is a receptor antagonist that competitively inhibits GTP signal transduction by excluding GTP from binding to its putative receptor. Such a receptor may even bind XTP more tightly than GTP, as suggested by XTP's low IC_{50} and the observation that equimolar XTP completely blocks the response to GTP (Fig. 1*B*).

Although XTP also completely blocks the oscillating current induced by GTP, it does not block the sustained current (Fig. 2). It is difficult to imagine how this observation can be accommodated into a linear model of GTP signal transduction in which GTP induces a sustained current, which in turn induces an oscillating current. If XTP is in fact a receptor antagonist, we feel that our observation can best be explained by a model in which GTP triggers two independent pathways. We therefore propose that *P. tetraurelia* possesses two separate purinergic signal transduction pathways, one of which produces an oscillatory current and repetitive backward swimming, while the other produces a sustained current and whirling. Although both pathways can be activated by GTP, only the oscillatory pathway can be blocked by XTP, by membrane permeant derivatives of cAMP, or by the *ginA* mutation.

The two-pathway hypothesis is supported by the finding that a sustained current can be activated by XTP alone in both wild type and in *ginA* mutant cells (Fig. 3), which respond to GTP with only a sustained current and whirling (Mimikakis, Nelson & Preston, 1998). Thus, whereas the oscillatory pathway is specifically activated by GTP, the nonoscillatory pathway may be caused by GTP or XTP. Are the oscillatory and nonoscillatory responses to purine nucleotides mediated by two distinct purinoreceptors, or does a single receptor give rise to a bifurcating pathway? Although a two-receptor model may seem more likely, other investigators have isolated a purinergic receptor from a vertebrate cell line that may have two distinct nucleotide binding sites and that responds differently to ATP and UTP (Czubayko & Reiser, 1996). Further investigation into the pharmacology of the two responses triggered by GTP and XTP in *P. tetraurelia* will likely help resolve the molecular basis for the two responses, and ultimately may help identify the purinergic receptor or receptors themselves.

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