J. Membrane Biol. 109, 251–258 (1989)

The Journal of
Membrane Biology

Veratridine Induces a Ca^{2+} Influx, Cyclic GMP Formation, and Backward Swimming in *Paramecium tetraurelia* Wildtype Cells and Ca^{2+} Current-Deficient Pawn Mutant Cells

Joachim E. Schultz and Uwe Schade
Pharmazeutisches Institut der Universität Tübingen, 7400 Tübingen, Federal Republic of Germany

Summary. Veratridine opens voltage-dependent Na^+ channels in many metazoans. In *Paramecium*, which has voltage-dependent Ca^{2+} channels and a Ca/K action potential, no such Na^+ channels are known. A Ca-inward current is correlated to an intracellular increase in cGMP. The addition of veratridine to *Paramecium* wildtype and to pawn mutant cells, which lack the Ca-inward current, transiently increased intracellular levels of cGMP about sevenfold to 40 pmol/mg protein. A half-maximal effect was obtained with 250 μM veratridine. The increase in cGMP was maximal about 15 sec after the addition of veratridine and declined rapidly afterwards. Intracellular cAMP levels were not affected. The effect of veratridine on cGMP was dependent on the presence of extracellular Ca^{2+}. The time dependence and extent of stimulation closely resembled the effects observed after stimulation by Ba^{2+}, which causes the repetitive firing of action potentials, Ca-dependent ciliary reversal, and cGMP formation. The effects of Ba^{2+} and veratridine were not additive. Wildtype cells and, surprisingly, also pawn mutant cells showed avoiding reactions upon addition of veratridine indicating that it induced a Ca^{2+} influx into the cilia, which causes ciliary reversal. The potency of veratridine to stimulate cGMP formation was little affected by Na^+ in wildtype cells, three pawn mutant strains, and in the cell line fast-2, which is defective in a Ca-dependent Na-inward current. Divalent cations (Ca^{2+}, Mg^{2+}, and Ba^{2+}) inhibited the effects of veratridine similar to metazoan cells. The results indicate that veratridine can open the voltage-operated Ca^{2+} channels in *Paramecium* wildtype and, most interestingly, in pawn mutant cells. The pawn mutation is suggested to represent a defect in the activation of the Ca^{2+} channel. This explains the lack of differences in ciliary proteins between wildtype and pawn cells reported earlier.

Key Words *mutant · veratridine · calcium channel · cyclic GMP*

Introduction

The unicellular protozoan *Paramecium* is much like a neuron with several ion channels differing in their properties and locations. The ion channels in *Paramecium* have been characterized by standard electrophysiological techniques (for review *see* [9]). A Ca-inward channel and a voltage-induced K-outward current generate a Ca/K action potential that lasts for several tens of milliseconds. If sodium ions are present in the bathing medium, the duration of the excited state can be extended to hundreds of milliseconds due to a depolarizing Ca-dependent Na-inward current [16]. Under these conditions, repolarization is effectively accomplished by a delayed, Ca-dependent K^+ current. Changes in the membrane potential are also reflected in the swimming behavior of *Paramecium*. Usually, when the membrane potential rests at about -30 mV, normal forward swimming is the prevailing mode of movement. Hyperpolarization due to an efflux of K^+ results in accelerated forward swimming, i.e., speeding. Depolarization caused by the transient influx of Ca^{2+} through voltage-operated Ca-channels triggers brief bouts of backward swimming, i.e., avoiding reactions. The dissection of individual currents and related behavior of *Paramecium* has been substantially aided by mutants in which different types of ion channels are seriously affected [17]. A group of mutants called pawn has a very small Ca-inward current. Consequently, these cells do not spontaneously generate Ca/K action potentials and do not reverse the ciliary beat. In contrast, the group of dancer mutants has a defect in the fast inactivation of the voltage-dependent Ca^{2+} channel. When stimulated, the Ca-inward current is much more persistent leading to a prolonged behavioral response, i.e., longer periods of backward swimming [7, 17]. In the past, we used these groups of Ca^{2+} channel mutants to demonstrate that the Ca-inward current generates an increase in intraciliary cGMP by a Ca-regulated guanylate cyclase [8, 19]. Thus, behavior and cGMP formation can be used as a convenient assay for Ca^{2+} channel function.

In *Paramecium* as a model organism, mutations can be extremely helpful to characterize the connection between a biochemical reaction and ion currents [19]. In vertebrate systems, such a genetic

approach is not possible. Instead, several toxins and drugs are used as pharmacological tools, which specifically interfere with ion channel function. Although the combined use of mutants and drugs should be of great advantage, most of these pharmacological compounds tested on *Paramecium* have little efficacy on specific ion conductances [9]. In this report, we present behavioral and biochemical evidence that the plant alkaloid veratridine, which opens Na channels in metazoan systems, is able to open Ca^{2+} channels in *Paramecium* wildtype cells. Furthermore, veratridine apparently can open nonfunctional or silent Ca^{2+} channels in pawn mutants with behavioral and biochemical consequences identical to those observed in wildtype cells.

Materials and Methods

Cell Cultures and Stimulation

Paramecium tetraurelia 51s, the mutants pawn A (d4-94), the double mutant pawn A/B, and fast-2 (d4-562) were axenically grown at 25°C—the temperature-sensitive pawn C (d4-131) at 35°C—in 1 liter Erlenmeyer flasks containing 300 ml medium with either phosphatidylcholine or phosphatidylethanolamine as major lipid source, respectively [22]. Early stationary cells were harvested by centrifugation (500 · *g* for 1 min) and transferred into 200 volumes of equilibration buffer (10 mM MOPS adjusted with Tris to pH 7.2, 1 mM KCl, 50 μM $CaCl_2$). During equilibration at 25°C (pawn C was equilibrated at 35°C), cells were put onto a rotary shaker (50 rpm). After at least 2 hr, cells were washed once with equilibration buffer and suspended to a final density of 20,000 cells/ml. All mutants were tested for their characteristic phenotype after equilibration.

Stock solutions of 50 mM veratridine (freshly dissolved for each experiment with 50 mM HCl) and 1 M $BaCl_2$ were diluted with equilibration buffer prior to use. For stimulation experiments, about 2500 cells in 125 μl buffer were mixed at room temperature of 21–23°C with an equal volume of stimulation solution. Unless otherwise indicated, incubations were stopped after 15 sec by addition of perchloric acid (1 M final).

Assays

cAMP and cGMP levels were assayed by a radioimmunoassay using ^{125}I-iodinated 2-O'-succinyl-cyclic nucleotide-L-tyrosinylmethylesters as tracers and respective antibodies against cAMP and cGMP with crossreactivities of less than 5% [3]. Protein precipitated by $HClO_4$ and pelleted by centrifugation was dissolved in 0.5 M NaOH and determined by the method of Lowry using bovine serum albumin as a standard. Swimming behavior of *Paramecium* was monitored in depression slides at room temperature under 10- to 30-fold magnification. Usually, several cells were observed at once and the response pattern prevailing in several experiments is listed. When necessary, additional experimental details are given in the figure legends.

Veratridine, tetrodotoxin, phosphatidylethanolamine and phosphatidylcholine were purchased from Sigma. All other chemicals were of analytical grade from usual sources.

Results

Behavioral Response of *Paramecium* to Veratridine

Avoiding reactions and continuous backward swimming of *Paramecium* indicate membrane depolarization of various duration. Usually, wildtype cells swim backward upon addition of Ba^{2+} if the concentration exceeds the Ca^{2+} concentration about fivefold [11]. This result is confirmed in the Table. Bouts of continuous backward swimming were observed on addition of 0.4 mM Ba^{2+} to the incubation medium containing 50 μM Ca^{2+}. Addition of veratridine to wildtype cells triggered a behavioral response very similar to that observed upon addition of Ba^{2+} (Table). This response was dependent on the presence of Ca^{2+} in the bathing medium, implicating a participation of external Ca^{2+} in the reaction cascade leading to ciliary reversal. The effect of veratridine was observed at slightly lower concentrations compared to Ba^{2+} (Table). 1 mM Veratridine was no more toxic to *Paramecium* than 1 mM Ba^{2+}. No signs of bodily damage or deterioration, e.g., blisters or lysis, were seen with 1 mM veratridine for at least 20 min. As expected, the mutants pawn A, pawn C, and pawn A/B completely lacked a behavioral response to Ba^{2+} since the voltage-operated Ca^{2+} channels are not functional in these cell lines (*see* Table for pawn A/B). Surprisingly, addition of veratridine to pawn mutant cells (pawn A, C, and pawn A/B) elicited a behavioral response indistinguishable from that of the wildtype (*see* Table for observations with pawn A/B). Since reversal of the ciliary beat requires the influx of Ca^{2+} through voltage-operated Ca^{2+} channels, which are localized in the ciliary membrane [4, 14], this result indicates that in pawn mutant cells veratridine activates a Ca^{2+} conductance, which is in close proximity to the mechanical switch for ciliary reversal.

Increase of cGMP in Response to Veratridine

Depolarization of *Paramecium* wildtype cells with Ba^{2+} transiently increases intracellular cGMP levels [7, 19]. Since veratridine elicited a backward swimming response of *Paramecium* similar to that of Ba^{2+}, we investigated the effect of veratridine on cGMP levels in vivo. Veratridine elicited a sevenfold increase in cGMP concentration within 15 sec (Fig. 1*A*). The time course displayed a maximum between 5 and 30 sec, thereafter the cGMP concentration declined and reached almost basal levels 4

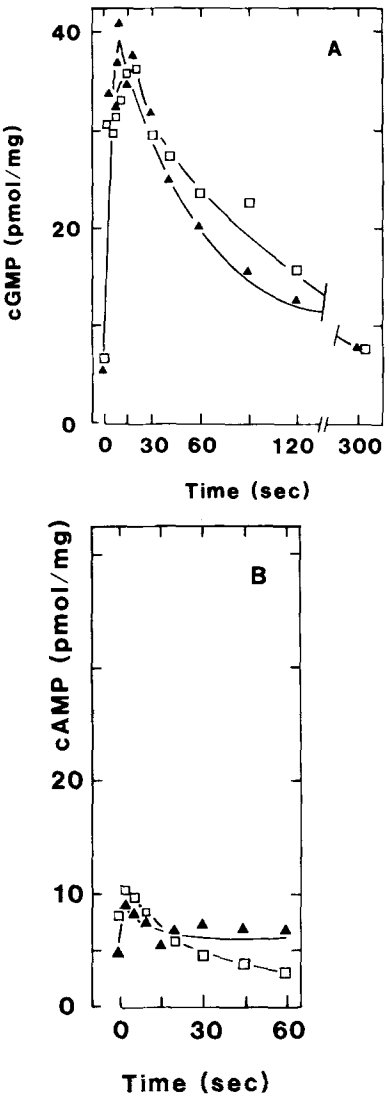

Fig. 1. (*A*) Formation of cGMP and (*B*) cAMP in *Paramecium tetraurelia* wildtype 51s after stimulation with 1 mM Ba^{2+} (□) or 1 mM veratridine (▲). In (*A*), mean values of three to six experiments are depicted (SEM 6%). In (*B*), one of two representative experiments is shown

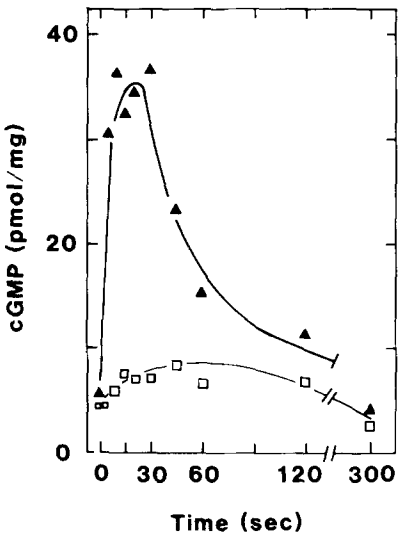

Fig. 2. Effect of veratridine on cGMP formation in the Ca^{2+} channel double mutant pawn A/B (▲: 1 mM veratridine; □: 1 mM Ba^{2+}). One of three representative experiments is shown

Table. Behavioral response of *Paramecium tetraurelia* wildtype 51s and double mutant pawn A/B to Ba^{2+} and veratridine[a]

Concentrations added (mM)	Wildtype 51s		Double mutant pawn A/B	
	Ba^{2+}	Veratridine	Ba^{2+}	Veratridine
0.1	Speeding	FS	FS	FS
0.2	FS	Tumble	FS	FS
0.4	CCR	CCR	FS	Tumble
0.6	CCR	CCR	FS	CCR
0.8	CCR	CCR	FS	CCR
1.0	Tumble	CCR	FS	CCR

[a] All cells were adapted to equilibration buffer containing 50 μM Ca^{2+} for at least 2 hr before transfer into test solutions. The behavior was monitored for 60 sec. Each entry into the table is derived from the results from at least 40 *Paramecia*.
FS = normal forward swimming mode; CCR = continuous ciliary reversal that results in prolonged and repeated backward swimming for tens to fifties of body lengths including repeated bouts of backward swimming lasting >1 sec in duration; tumble = the cell tumbles or rotates around its posterior end, either remaining in place or moving very slowly forward.

min later (Fig. 1*A*). The time course of the cGMP response to veratridine was comparable to the response to 1 mM Ba^{2+} (Fig. 1*A*). Neither Ba^{2+} [8, 19] nor veratridine enhanced ciliary guanylate cyclase activity in vitro. In fact, veratridine concentrations exceeding 100 μM inhibited guanylate cyclase activity (*data not shown*). Therefore, it is conceivable that veratridine enhanced cGMP formation by an influx of Ca^{2+} through voltage-operated Ca^{2+} channels similar to a Ba^{2+} stimulation. This hypothesis is supported by the following observations. First, the cGMP elevation elicited by veratridine required the presence of Ca^{2+} in the external medium. In the presence of 5 nM Ca^{2+} (adjusted by EGTA addition) during the stimulation, no increase of cGMP was found (*data not shown*). Secondly, like Ba^{2+}, veratridine specifically stimulated cGMP formation while the intracellular cAMP concentration remained almost unchanged (Fig. 1*B*).

In pawn mutant cells, Ba^{2+} addition has little effect on cGMP levels, probably due to the large reduction of the Ca-inward current (Fig. 2; [19]).

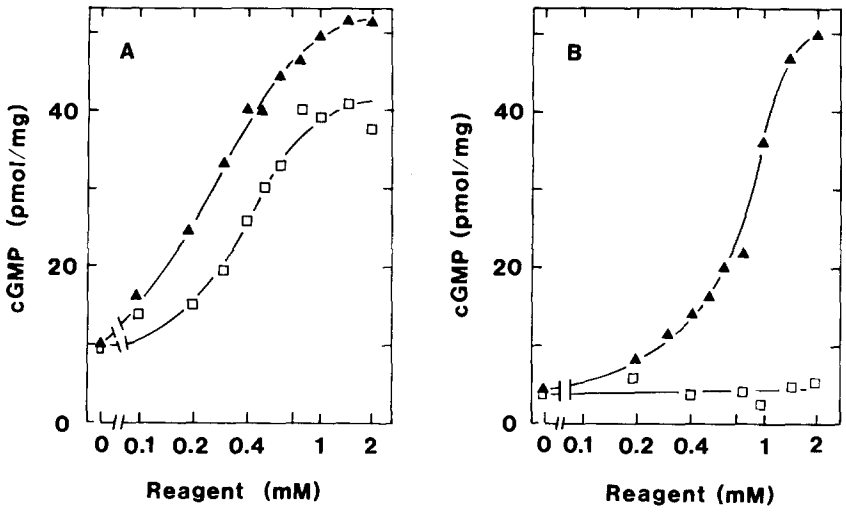

Fig. 3. Dose-response curves for Ba^{2+} or veratridine-stimulated cGMP formation in *Paramecium tetraurelia* (*A*) wildtype 51s or (*B*) pawn A/B mutant cells. In (*A*), mean values of 24 (Ba^{2+}, □) or seven (veratridine, ▲) experiments are shown (SEM <5%). In (*B*), a representative experiment is depicted ($n = 2$ for Ba^{2+}; $n = 4$ for veratridine). Stimulation time in this and following figures was 15 sec

However, all pawn mutants tested with veratridine showed a strong backing response indicating a functional Ca-inward current. When cGMP levels were measured in pawn A, pawn C*ts*, and the double mutant pawn A/B after stimulation with veratridine, a response like that in wildtype cells was observed (*see* Fig. 2 for data with pawn A/B). Obviously, veratridine stimulates a Ca-inward current in pawn mutant cells, which is functionally coupled to both ciliary reversal and cGMP formation. It should be noted that the onset of the decline in cGMP levels in wildtype and pawn mutant cells 15 sec after veratridine addition coincided with an adaptive normalization of the behavioral response, which is usually observed after addition of moderate concentrations of Ba^{2+} to wildtype cells.

Dose-Response Curves for Veratridine

An initial effect of veratridine on the behavior of *Paramecium* was apparent with 200 μM. We established a dose-response curve for cGMP formation elicited by veratridine and Ba^{2+} (Fig. 3*A*). At all concentrations, the plant alkaloid was slightly more potent than Ba^{2+}. A small increase in cGMP was already found with 100 μM veratridine, the concentration for a half maximal effect (EC_{50}) was 250 μM ($n = 7$, range 180–400 μM). The EC_{50} for Ba^{2+} was 400 μM under identical experimental conditions. When the mutant pawn A/B was stimulated with up to 2 mM Ba^{2+}, no cGMP elevation was found (Fig. 3*B*). With pawn cells, the dose-response curves of veratridine were slightly shifted to higher doses compared to wildtype cells. The EC_{50} concentrations were 400 μM for pawn A and C, and 700 μM ($n = 4$, range 450–900 μM) for the double mutant pawn A/B (Fig. 3*B*).

Effect of Cations on Veratridine Stimulation

In metazoans, veratridine persistently activates voltage-gated Na^+ channels [2, 24]. In *Paramecium*, a voltage-dependent Na^+ channel is not present [9]. The only identified Na^+ conductance in this protozoan is a Ca-dependent Na-inward current, which can be measured in the presence of Na^+ in the medium [7, 9]. The Ca-dependent Na-inward current enhances the effect of depolarizing stimuli. This Na^+ conductance of *Paramecium* is increased in the group of paranoiac mutants and almost absent in the fast-2 mutants [7, 15]. In the experiments so far, Na^+ was not added. Therefore, an effect of veratridine on a Na^+ conductance would have gone unnoticed. Now we equilibrated *Paramecium* in a buffer containing 10 mM Na^+ and investigated whether the stimulatory effect of veratridine on intracellular cGMP formation was altered. 10 mM Na^+ generally attenuated the potency of veratridine (Fig. 4). While Na^+ slightly shifted the dose-response curve to the right, it did not impair the maximal response. The effect was identical for wildtype cells (Fig. 4*A*), the mutant fast-2, which lacks the Ca-dependent Na^+ current [15] (Fig. 4*B*), and for the double mutant pawn A/B, which is devoid of the voltage-dependent Ca-inward current (Fig. 4*C*). As

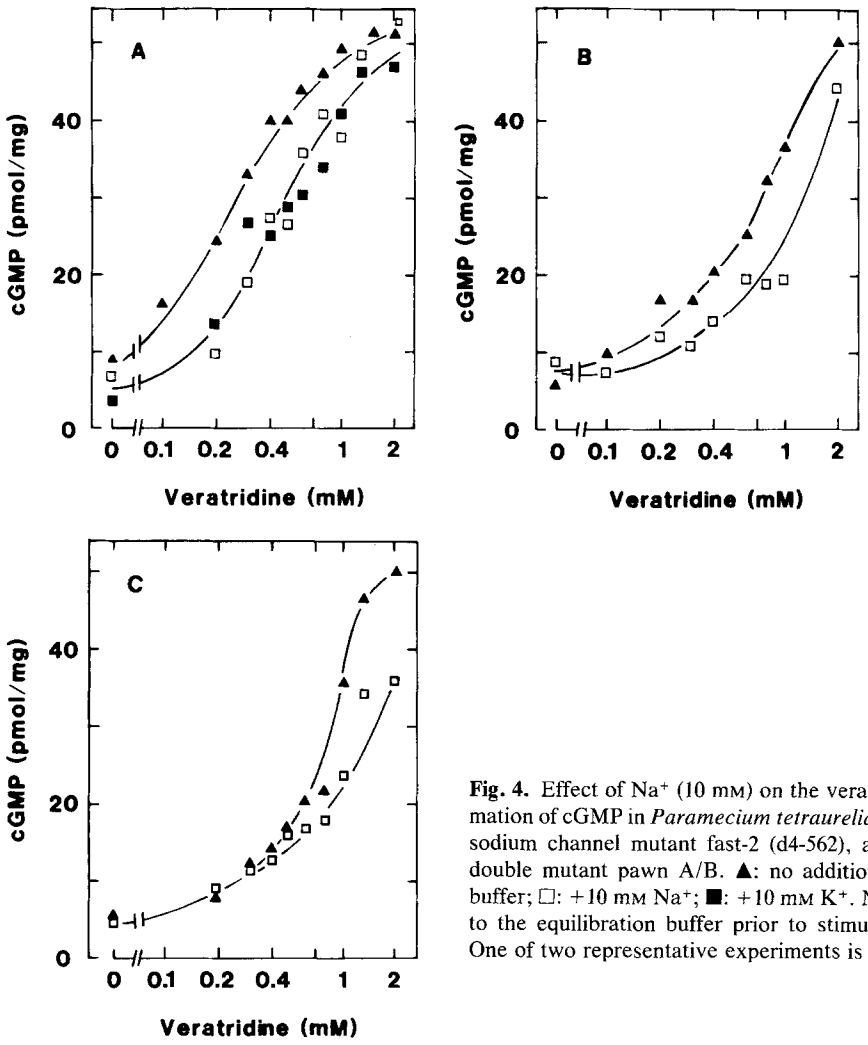

Fig. 4. Effect of Na^+ (10 mM) on the veratridine-stimulated formation of cGMP in *Paramecium tetraurelia* (*A*) wildtype 51s, (*B*) sodium channel mutant fast-2 (d4-562), and (*C*) Ca^{2+} channel double mutant pawn A/B. ▲: no addition to the equilibration buffer; □: +10 mM Na^+; ■: +10 mM K^+. Na^+ or K^+ were added to the equilibration buffer prior to stimulation by veratridine. One of two representative experiments is shown

a control, we increased the K^+ concentration in the equilibration buffer instead of Na^+. The potency of veratridine was slightly diminished (Fig. 4*A*). This demonstrated that in *Paramecium* veratridine most likely had no specific effect on a Na^+ conductance. The small attenuation of activity by Na^+ and K^+ probably was due to an unspecific interaction with surface charges of *Paramecium*. Furthermore, the effect of 800 μM veratridine was not antagonized by 100 μM tetrodotoxin (*data not shown*), which in most tissues counteracts veratridine [10]. In metazoans, the activation of Na^+ channels by veratridine is often inhibited by divalent cations [2, 24]. This was also observed in *Paramecium*. 10 mM Ca^{2+} completely blocked the cGMP increase in wildtype cells, which were stimulated by up to 2 mM veratridine (Fig. 5*A*). The specificity of this inhibition was further investigated using pawn A/B cells since Ba^{2+} could be used with this mutant. Unlike in wildtype cells, Ba^{2+} does not cause an elevation of cGMP levels in this mutant (*see* Fig. 2). As already reported for Na^+ channel activation in metazoan systems [2], Ca^{2+} was more potent than Mg^{2+} and Ba^{2+} in inhibiting the veratridine effect in *Paramecium* (Fig. 5*B*). These results stress that the mechanism of action of veratridine in *Paramecium* resembled in several respects the situation in higher systems.

Discussion

First we address the question whether veratridine stimulates cGMP formation in *Paramecium* by directly affecting the enzymes of cGMP metabolism or by indirectly stimulating an influx of Ca^{2+}. Control experiments show that veratridine does not activate the guanylate cyclase or inhibit a phosphodiesterase from *Paramecium* in vitro (*data not shown*). If any, veratridine inhibits guanylate cyclase activity at concentrations exceeding 100 μM.

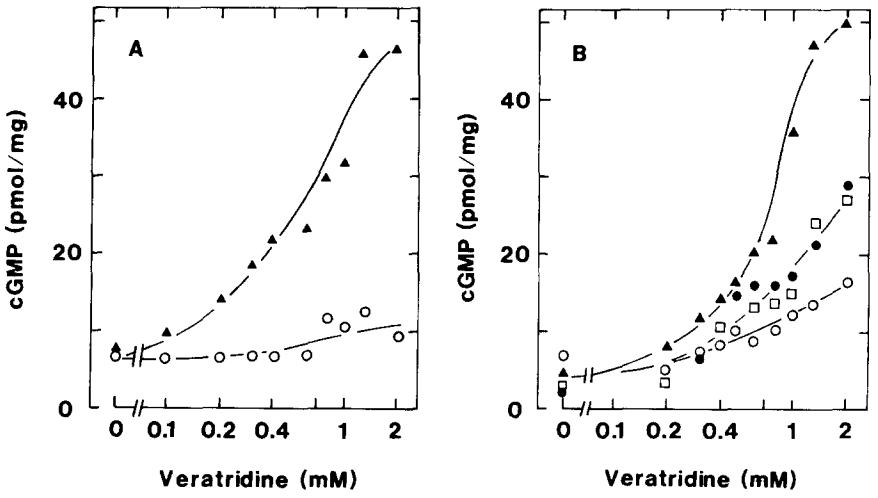

Fig. 5. Antagonism of the actions of veratridine by divalent cations. *Paramecium* (*A*) wildtype 51s; (*B*) pawn A/B. Symbols are: ▲: no addition; ○: +1 mM Ca^{2+}; ●: +1 mM Ba^{2+}; □: +1 mM Mg^{2+}. Veratridine and the respective divalent cations were added concomitantly. For clarity of presentation, not all points measured with Mg^{2+} and Ba^{2+} are depicted in (*B*). One of two representative experiments is shown

The presence of Ca^{2+} in the bathing solution of *Paramecium* was absolutely required for the effect of veratridine on cGMP levels and behavior to occur. We conclude that the entry of Ca^{2+} into *Paramecium* caused by the plant alkaloid is responsible for activation of the Ca-regulated guanylate cyclase and for the backward swimming response.

The next question to be considered is whether the Ca^{2+} translocation triggered by veratridine occurred by a direct activation of the Ca^{2+} conductance in *Paramecium* or by another hitherto unknown mechanism. Veratridine is best known to cause persistent activation of Na^+ channels in electrically excitable cells from metazoans, an action on protozoans has not yet been tested. It would be conceivable that veratridine caused a depolarization of *Paramecium* by stimulating a Na^+ influx, which could in turn activate voltage-operated Ca^{2+} channels. However, the experimental evidence does not support this idea. First of all, a voltage-operated Na^+ channel has not been found in *Paramecium*. A Ca-dependent Na-inward current is known to occur only in the presence of Na^+ in the bathing solution. However, the effects of veratridine were little influenced by the presence or absence of Na^+. In addition, tetrodotoxin, a powerful inhibitor of the action of veratridine on Na^+ channels, was not inhibitory irrespective of the presence of Na^+. Although a participation of a Na^+ current in the actions of veratridine in *Paramecium* should not be completely discarded, it is highly unlikely and we are left with the conclusion that veratridine directly stimulates a Ca^{2+} influx. Most likely, the Ca-inward current did not occur through the Ca-dependent Na^+ channel of *Paramecium*, which could have lost their ion selectivity due to veratridine because the fast-2 mutant, which lacks this current [15], was equally affected by veratridine as wildtype cells. Because veratridine itself is no Ca^{2+} ionophor and is not known to unspecifically permeabilize cells at the concentrations used in this study, we believe that the experimental data indicate that Ca^{2+} enters *Paramecium* via an established channel for divalent cations. So far, three such channels have been identified: (*i*) the anterior mechanoreceptor channel [13, 21], (*ii*) a hyperpolarization-activated Ca^{2+} channel [15], and (*iii*) the voltage-gated Ca^{2+} channel of the ciliary membrane. First, in pawn mutant cells, the depolarizing mechanoreceptor potential caused by Ca^{2+} influx is quite normal [21], yet, these cells are unable to swim backward. Obviously, the small mechanoreceptor-activated Ca^{2+} current alone is not sufficient to cause ciliary reversal. Mechanical stimulation of cells also does not elicit cGMP formation [19]. Because the backward swimming and cGMP response of pawn cells toward veratridine is identical compared to wildtype *Paramecium*, the plant alkaloid is unlikely to act only on the anterior mechanoreceptor channel. Furthermore, it is difficult to envision that in *Paramecium* veratridine will only affect a highly specialized mechanoreceptor-operated channel for divalent cations. Second, a hyperpolarization-activated Ca^{2+} current has so far been observed under voltage clamp at potentials smaller than −50 mV. At −90 mV, the size of this current is about one-third compared with the depolarization-activated current [15]. *Paramecium* characteristically responds to hyperpolarization with forward speeding. This was not observed upon veratridine addition. Although

we cannot completely rule out the possibility that veratridine shifted the voltage sensitivity of the hyperpolarization-activated channel to more positive potentials, we consider this rather unlikely. Under these circumstances, the cellular localization of the hyperpolarization-gated channel would have to be in close proximity to the site of ciliary reversal and the Ca-dependent guanylate cyclase, similar to what is known for the voltage-gate channel [4, 14, 19].

Although electrophysiological studies are lacking and are beyond the scope of this paper, we believe that our data are most plausibly explained by the hypothesis that veratridine directly promotes Ca^{2+} entry through the voltage-operated Ca^{2+} channels of *Paramecium*. Indeed, the influx of Ca^{2+} must occur rapidly and at the same site as that triggered by other depolarizing stimuli, i.e., at the ciliary membrane, which carries Ca^{2+} channels and guanylate cyclase. Ciliary reversal and cGMP increase occur concomitantly and are reliable indicators of Ca^{2+} channel function. The Ca-inward current shuts off rather rapidly as visualized by the normalization of the behavior and the fast decline of cGMP levels after 30 sec. After a veratridine stimulation, the Ca^{2+} channels are in an inexcitable inactivated state and are reactivated to an excitable closed state in a time- and temperature-dependent process [20]. Depolarizations elicited by concomitant additions of 10 mM Na^+, 10 mM K^+, or 1 mM Ba^{2+} together with 1 mM veratridine were not additive or synergistic above the maximal levels obtained with either Ba^{2+} or veratridine alone. If the two agents would act through different channel types, then the responses evoked should have been additive. Taken together, these findings indicate that the two excitatory agents, Ba^{2+} and veratridine, use the same channel. Considering that veratridine activates Na^+ channels in metazoan cells, it is reasonable to suggest [5] that the protozoan Ca^{2+} channel may be an evolutionary precursor of the voltage-operated Na^+ channel. In fact, voltage-operated Na^+ channels insensitive to tetrodotoxin are known to exist in higher organisms [5].

The results with the pawn mutants were surprising and puzzling at first. Usually, pawns provide null controls in experiments in which the flow of Ca^{2+} or its consequences are studied. However, essentially all pawn cell lines tested electrophysiologically (with 1 mM Ca^{2+} in the bathing fluid) display different degrees of leakiness indicating that at least a very low Ca^{2+} conductance is still functional [e.g., 11, 18, 23]. Using wildtype cells, it was found with electrophysiological methods that the voltage-operated Ca^{2+} channels of *Paramecium* are localized in the ciliary membrane [4, 14]. Therefore, a great effort was made using one- and two-dimensional gel electrophoresis to identify bands possibly related to a Ca^{2+} channel protein by comparisons of wildtype and pawn membrane proteins [1, 12]. However, no reproducible differences were observed [1]. The data presented here provide a simple explanation for this failure.

Functionally defective Ca^{2+} channels are present in the pawn mutants studied here: pawn A, temperature-sensitive pawn C, and the least leaky double mutant pawn A/B. The plant alkaloid veratridine was able to activate these Ca^{2+} channels, although at somewhat lower potency compared to wildtype cells. Behavioral reactions were initiated, which were identical to wildtype *Paramecia,* and veratridine stimulation of intracellular cGMP formation in all pawns was kinetically and quantitatively indistinguishable from that observed in wildtype cells. The most plausible interpretation is that pawns carry a defect in the activation of the Ca^{2+} channel. While veratridine is able to activate the Ca^{2+} channels despite the mutation, Ba^{2+} is unable to do so under standard incubation conditions. In fact, at 50 μM Ca^{2+} in the bathing medium, Ba^{2+} at higher concentrations also induced a weak behavioral response and a cGMP increase in leaky pawns (*data not shown*). The suggested mutation in pawn, a reduction of the activation of the Ca^{2+} channel, is contrary to the dancer mutation, which is a reduction in the inactivation of the Ca^{2+} channel [6, 7]. The existence of different genetic loci in several pawn isolates is not contradictory to the interpretation of our results, since the mutations may be distal to the actual channel protein. In fact, the findings permit a novel look at the pawn mutation. With the channel protein itself present in the ciliary membrane, biochemical processes involved in activation/inactivation cycles of the channel may gain an increased importance. Extensive electrophysiological studies using veratridine should further reveal the nature of the mechanism of action of veratridine and give more insight concerning the defect of the pawn mutation.

We thank Dr. C. Kung, University of Wisconsin, Madison, for providing the *Paramecium* mutants and Drs. U.B. Kaupp, Jülich, and G. Reiser, Tübingen, for critical comments on the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 323).

References

1. Adoutte, A., Ling, K.-Y., Chang, S., Huang, F., Kung, C. 1983. Physiological and mutational protein variations in the ciliary membrane of *Paramecium*. *Exp. Cell Res.* **148**:387–404
2. Catterall, W.A. 1975. Activation of the action potential Na^+ ionophor of cultured neuroblastoma cells by veratridine and batrachotoxin. *J. Biol. Chem.* **250**:4053–4059

3. Delaage, M.A., Roux, D., Cailla, H.L. 1978. Molecular Biology and Pharmacology of Cyclic Nucleotides. G. Falco, R. Paoletti, editors. pp. 155–170. Elsevier, Amsterdam
4. Dunlap, K. 1977. Localization of calcium channels in *Paramecium* caudatum. *J. Physiol. (London)* **271:**119–133
5. Hille, B. 1984. Ionic Channels in Excitable Membranes. Chap. 16. Sinauer Associates, Sunderland, Massachusetts
6. Hinrichsen, R.D., Saimi, Y., Kung, C. 1984. Mutants with altered Ca^{2+}-channel properties in *Paramecium tetraurelia:* Isolation, characterization and genetic analysis. *Genetics* **108:**545–558
7. Hinrichsen, R.D., Schultz, J.E. 1988. *Paramecium:* A model system for the study of excitable cells. *Trends Neurosci.* **11:**27–32
8. Klumpp, S., Schultz, J.E. 1982. Characterization of a Ca^{2+}-dependent guanylate cyclase in the excitable ciliary membrane from *Paramecium. Eur. J. Biochem.* **124:**317–324
9. Kung, C., Saimi, Y. 1985. Ca^{2+} channels of *Paramecium:* A multidisciplinary study. *Curr. Top. Membr. Transp.* **23:**45–66
10. Leibowitz, M.D., Sutro, J.B., Hille, B. 1986. Voltage-dependent gating of veratridine-modified Na^+ channels. *J. Gen. Physiol.* **87:**25–46
11. Ling, K.-Y., Kung, C. 1980. Ba^{2+} influx measures the duration of membrane excitation in *Paramecium. J. Exp. Biol.* **884:**73–87
12. Merkel, S.J., Kaneshiro, E.S., Gruenstein, E.I. 1981. Characterization of cilia and ciliary membrane proteins of wild-type *Paramecium tetraurelia* and a pawn mutant. *J. Cell. Biol.* **89:**206–215
13. Ogura, A., Machemer, H. 1980. Distribution of mechanoreceptor channels in the *Paramecium* surface membrane. *J. Comp. Physiol.* **135:**233–244
14. Ogura, A., Takahashi, K. 1976. Artificial deciliation causes loss of calcium-dependent responses in *Paramecium. Nature (London)* **268:**170–172
15. Saimi, Y. 1986. Calcium-dependent sodium currents in *Paramecium:* Mutational manipulations and effects of hyper- and depolarization. *J. Membrane Biol.* **92:**227–236
16. Saimi, Y., Hinrichsen, R.D., Forte, M., Kung, C. 1983. Mutant analysis shows that the Ca-induced K^+ current shuts off one type of excitation in *Paramecium. Proc. Natl. Acad. Sci. USA* **80:**5112–5116
17. Saimi, Y., Kung, C. 1987. Behavioral genetics of *Paramecium. Annu. Rev. Genet.* **21:**47–65
18. Satow, Y., Kung, C. 1980. Membrane currents of pawn mutants of the pwA group in *Paramecium tetraurelia. J. Exp. Biol.* **84:**57–71
19. Schultz, J.E., Pohl, T., Klumpp, S. 1986. Voltage-gated Ca^{2+} entry into *Paramecium* linked to intraciliary increase of cyclic GMP. *Nature (London)* **322:**271–273
20. Schultz, J.E., Schade, U. 1989. Calcium channel activation and inactivation in *Paramecium* biochemically measured by cyclic GMP production. *J. Membrane Biol.* **109:**259–267
21. Takahashi, M., Haga, N., Hennessey, T. M., Hinrichsen, R.D., Hara, D. 1985. A gamma ray-induced non-excitable membrane mutant in *Paramecium caudatum:* A behavioral and genetic analysis. *Genet. Res.* **46:**1–10
22. Thiele, J., Honer-Schmid, O., Wahl, J., Kleefeld, G., Schultz, J.E. 1980. A new method for axenic mass cultivation of *Paramecium tetraurelia. J. Protozool.* **27:**118–121
23. Thiele, J., Otto, M.K., Deitmer, J.W., Schultz, J.E. 1983. Calcium channels of the excitable membrane from *Paramecium:* An initial biochemical characterization. *J. Membrane Biol.* **76:**253–260
24. Ulbricht, W. 1969. The effect of veratridine on excitable membranes of nerve and muscle. *Ergeb. Physiol. Biol. Chem. Exp. Pharmakol.* **61:**18–71

Received 8 December 1988; revised 16 March 1989