Serotonergic Stretch Receptors Induce Plateau Properties in a Crustacean Motor Neuron by a Dual-Conductance Mechanism

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SUMMARY AND CONCLUSIONS

1. The mechanisms for induction of bistable plateau potential properties by a set of serotonergic/cholinergic peripheral stretch receptor cells [gastropyloric receptor (GPR) cells] were examined in the crab stomatogastric ganglion (STG) with the use of intracellular recording techniques.

2. GPR cell stimulation evoked nicotinic excitatory postsynaptic potentials (EPSPs) and induced plateau potential capability in the dorsal gastric (DG) motor neuron. The plateau potential could be triggered during a GPR train either by the summating nicotinic EPSPs or by brief intracellular current injection. After pharmacological blockade of nicotinic and muscarinic receptors, a slow depolarization in response to GPR stimulation was revealed. Prolonged plateau potentials could still be evoked after this treatment. Local application of serotonin (5-HT; 10 μM to 1 mM) mimicked the noncholinergic plateau inducing effects of GPR stimulation in the DG motor neuron.

3. The synergistic action of acetylcholine (ACh) and 5-HT was examined by stimulating the GPR cells at different frequencies (1-20 Hz). The plateau induction was present down to 2 Hz. The time to onset for triggering a plateau during a GPR train was determined by the co-released ACh.

4. The 5-HT-evoked slow depolarization persisted in tetrodotoxin (TTX; 0.1-1 μM), and the DG motor neuron could still produce a plateau potential on brief depolarization in the absence of the spike-generating mechanism.

5. In normal TTX-containing saline, the 5-HT-evoked depolarization was accompanied by a weak and variable decrease in apparent input conductance. After substituting one-half of the extracellular sodium with either Trisma-HCl or choline, the decrease in apparent input conductance became more pronounced. This decrease was converted to an increase in apparent input conductance when extracellular Ca2+ was replaced with Mg2+.

6. Under voltage-clamp conditions, local application of 5-HT caused a slow inward current of prolonged duration in the DG. The current versus voltage relationship had an inverted U-shape with a conductance increase from hyperpolarized voltages and a conductance decrease from moderately depolarized voltages.

7. Extracellular Cs+ (2-4 mM) caused the DG to hyperpolarize 2-4 mV from rest, whereas lowering extracellular Ca2+ caused it to depolarize 7-15 mV. The combined action of low extracellular Ca2+ and 2-4 mM Cs+ caused an almost complete block of the slow 5 HT-evoked depolarization. These results suggest that modulation of a cesium-sensitive and a calcium-dependent current are responsible for the 5-HT-evoked depolarization and that both conductances contribute to the resting membrane potential in DG.

8. In current-clamp experiments, the noncholinergic GPR activity caused a decrease in apparent input conductance in DG when the cell was relatively depolarized and an apparent increase in input conductance when the cell was relatively hyperpolarized.

9. A combined conductance increase and decrease was seen in response to both GPR stimulation and 5-HT application, indicating that it provides at least a partial mechanism for how plateau potentials are released in DG. These results reveal a unique mechanism by which neurotransmitters released from peripheral sources change motor neuron excitability. In an accompanying paper we have identified two target conductances underlying this conductance increase and decrease mechanism.

INTRODUCTION

A number of studies have shown that active membrane properties, such as bistable plateau potential capability and rhythmic bursting properties, are important elements of neuronal information processing in both invertebrate and vertebrate motor systems (Chrachri and Clarac 1990; Conway et al. 1988; Grillner and Wallén 1985; Grillner et al. 1987; Harris-Warrick and Flamm 1987; Hartline et al. 1988; Hounsgaard et al. 1988; Hounsgaard and Kiehn 1989; Plummer and Kirk 1990; Russell and Hartline 1978, 1984; Schwindt and Crill 1980a,b; Selverston and Moulins 1987). Plateau and bursting properties provide mechanisms for amplification of synaptic inputs and a source for repetitive outputs independent of ongoing synaptic activity (Hartline et al. 1988; Kiehn 1991; Selverston and Moulins 1987). If present in either motor neurons or neuronal components of motor networks, such properties, which constitute a type of intrinsic neuronal programming, will dramatically affect both rhythmic and static motor behavior, including changes in phase relationships and in the frequency and intensity of motor output (Hartline et al. 1988; Kiehn 1991; Selverston and Moulins 1987). It is therefore of importance to study these properties in the context of motor systems.

In most of the motor systems studied to date, plateau and bursting properties are dependent on modulatory inputs (Dickinson and Nagy 1983; Harris-Warrick 1988; Harris-Warrick and Flamm 1987; Hounsgaard et al. 1988; Russell and Hartline 1978, 1984). Neurotransmitters such as monoamines (Harris-Warrick 1988; Harris-Warrick and Flamm 1987; Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988), peptides (Marder 1987), and excitatory amino acids (Engberg et al. 1984; Grillner and Wallén 1985; Grillner et al. 1987) have been identified as putative transmitter candidates for the induction of plateau and bursting properties. Most experimenters have studied plateau induction after exogenous application of the compounds. How-
ever, to fully understand the physiological role of these compounds, it is necessary to study their effects under more natural conditions where the transmitter is released from presynaptic terminals.

The stomatogastric nervous system is a small and well-defined motor system controlling the movement of the foregut in decapod crustaceans and provides an excellent model for such studies. Here, several types of identified neuromodulatory neurons have been shown to induce rhythmic membrane potential oscillations or bistable plateau potentials in neurons located in the stomatogastric ganglion (STG) (see Katz and Harris-Warrick 1990a for references). Plateau properties are very important for the neuronal processing in STG neurons, but, like in other systems, very little is known about the actual ionic changes underlying the transmitter-regulated plateau induction (Hartline et al. 1988; Kiehn 1991). Among the modulatory neurons is a set of four peripheral sensory cells, the gastropyloric receptor cells (GPR), which are stretch-activated neurons in the foregut of the crab *Cancer borealis* (Katz et al. 1989; Katz and Harris-Warrick 1989, 1990b). These cells use both ACh and serotonin (5-hydroxytryptamine, 5-HT) as cotransmitters and project to the STG. In a particular motor neuron, the dorsal gastric (DG) motor neuron, stimulation of the GPR cells evokes rapid nicotinic excitatory postsynaptic potentials (EPSPs) and induces prolonged plateau potentials (Katz and Harris-Warrick 1989).

The DG motor neuron in the crab STG therefore provides an excellent model for studying the ionic mechanisms for plateau induction under conditions of natural transmitter release as well as interactions between co-released transmitters. We have begun this study by showing that 5-HT can induce plateau potential capabilities in DG. We also demonstrate that GPR activity and 5-HT cause excitation of the DG motor neuron by a mixed conductance increase and decrease mechanism. This unique mechanism is at least partially responsible for the GPR-evoked plateau induction and burst enhancement in DG. In the accompanying paper (Kiehn and Harris-Warrick 1992) we identify two ionic currents whose conductances are regulated by 5-HT in the DG motor neuron. Part of these data have appeared in abstract form (Kiehn and Harris-Warrick 1990).

**METHODS**

**Animals**

Jonah crabs (*C. borealis*) were obtained from local suppliers in Massachusetts and kept at 12-14°C in artificial seawater.

**Anatomy**

All experiments were performed in the isolated stomatogastric nervous system, which consists of the paired commissural ganglion (COG, Fig. 1), the oesophageal ganglion (OEG), and STG. The STG is connected anteriorly to the rest of the nervous system through the stomatogastric nerve (stn); it contains ~30 neurons, most of which are motor neurons. The motor axons exit mainly posteriorly and project bilaterally through various motor nerves to their respective muscles.

The projection pattern of the GPR cells is shown in Fig. 1. There are two GPR cell types, GPR1 and GPR2, that are distinguished by their muscle innervation and physiological response characteristics (Katz et al. 1989). Each GPR type exists as a bilaterally symmetrical pair; the right pair of cells is shown in Fig. 1. GPR cells project to the STG, where they arborize extensively before projecting to the COG (Katz et al. 1989). In the crab the GPR cells provide the only 5-HT innervation of the STG.

**General physiology**

After dissection of the stomatogastric nervous system (see Selverston et al. 1976) it was placed in a silicone elastomer (Sylgard)-lined Petri dish and superfused with cold (13-16°C) oxygenated solution (composition in mM: 440 NaCl, 11 KCl, 13 CaCl₂, 26 MgCl₂, 8 glucose, 11 Trizma base, 5 maleic acid, pH 7.4). The STG was surrounded by a petroleum jelly (Vaseline) barrier, creating a small central chamber for the ganglion. Owing to the small volume of this chamber (0.3-0.5 ml), rapid equilibration of drugs was obtained. Extracellular recordings from nerves were made with platinum pin electrodes or bipolar suction electrodes. Motor neurons in the STG were impaled with glass microelectrodes and identified by a one-for-one correlation of the intracellularly recorded action potentials with spikes recorded extracellularly in the appropriate motor nerve.

After identification of the GPR cells as described previously (Katz et al. 1989; Katz and Harris-Warrick 1989), a Vaseline pool on the stn (Fig. 1) was filled with an isotonic sucrose solution in 0.1 M Tris maleate alone or with 1 μM tetrodotoxin (TTX) added. The sucrose block was maintained for at least 1 h before experiments were started. This functionally isolated the STG from descending modulatory inputs from anterior ganglia and also prevented the GPR cells from acting indirectly on the STG via cells in the anterior ganglia (see Fig. 1). The latter point is important because we were interested in investigating only the direct effects of GPR stimulation on neurons in the STG.

**Stimulation**

Single GPR cells were stimulated extracellularly near their somata as previously described (Katz and Harris-Warrick 1989). These neurons are located in fine nerve branches that also contain axons of identified STG motor neurons. Careful placement of the stimulating electrode resulted in the GPR cell being the lowest
threshold unit. Contralateral motor nerves were monitored to ensure that this stimulation did not directly excite the motor neurons running in the same nerves. To examine the GPR effects on the DG motor neuron, we stimulated a GPR cell with square pulses (duration, 1 ms) in trains of 1–20 Hz, corresponding to firing frequencies observed in GPR cells in the semi-intact foregut (Katz et al. 1989). The GPR cell types were stimulated indiscriminately, because their effects on STG neurons are not qualitatively different.

**RESULTS**

**Pressure ejection of 5-HT mimics the noncholinergic effects of GPR stimulation on the DG motor neuron**

Katz and Harris-Warrick (1989) showed that GPR activity had dual effects on the DG motor neuron. First, GPR stimulation at low frequency (1 Hz) evoked discrete rapid EPSPs, with a typical nicotinic profile. Second, brief trains of stimulation of a GPR cell induced plateau properties in DG. During the GPR train the nicotinic EPSPs summated (Fig. 2A, top trace, \( \uparrow \)), and when the depolarization reached a certain voltage threshold (Fig. 2A), the membrane potential jumped to a depolarized voltage plateau with high-frequency spike activity. The duration of the plateau and associated spike train was dependent on the holding potential (not shown) and intensity of GPR stimulation (Fig. 3, D and E). If the spike train failed to trigger the plateau, it could be triggered by a brief depolarizing current pulse injected into the cell (Fig. 2A, A and B). The plateau eventually terminated spontaneously (Fig. 2B) or could be prematurely terminated by a brief hyperpolarizing pulse or synaptic inhibition.

The ability of GPR to induce plateau potentials persists after blocking the rapid EPSPs with nicotinic (and muscarnic) antagonists (Katz and Harris-Warrick 1989), which suggests that the plateau induction is not due to release of ACh. This is illustrated in Fig. 2C, where, in the presence of 0.1 mM \( \delta \)-tubocurarine and 0.1 mM atropine, a slowly developing depolarization \( \downarrow \) still followed the GPR train (10 Hz), and the cell fired a plateau potential in response to a brief depolarizing current injection. In other preparations the plateau was triggered during the GPR stimulation, the main difference being that the time for reaching the voltage threshold was delayed after suppressing the nicotinic EPSPs (Fig. 2C).

Katz and Harris-Warrick (1989) noted that bath application of 5-HT caused the DG motor neuron to depolarize and spike tonically at high frequency, but without displaying plateau properties. This left the possibility that another as yet unidentified transmitter was responsible for the GPR-evoked plateau properties in DG. We have now reexamined the 5-HT effect on DG and shown that the GPR-evoked nonnicotinic plateau induction can be mimicked by a short (0.5–2 s) puff of 5-HT (10 \( \mu \)M to 1 mM; \( n = 9 \)) onto the neuropil from a nearby pipette. Before pressure ejection 5-HT onto the neuropil, short-lasting current injections (Fig. 2D, bottom trace) of different amplitude caused a marked depolarization and spiking in DG, which, however, did not outlast the period of current injection. After the puff of 5-HT, a slow depolarization developed \( \uparrow \) and a short current injection now triggered a plateau potential with high-frequency spike, which far outlasted the period of current injection. The plateau terminated spontaneously (Fig. 2D) or could be terminated by a brief hyperpolarizing pulse.
Serotonin (5-HT) mimics the noncholinergic gastropyloric receptor (GPR) cell-evoked plateau induction in the dorsal gastric (DG) motor neuron. A: a train of GPR stimuli (10 Hz; indicated by the bar above the current trace) evoked a depolarization in DG (top trace), due in part to summation of fast nicotinic excitatory postsynaptic potentials (see text). This depolarization triggered a prolonged plateau potential, with high-frequency spike activity. Depolarization alone (induced by current injection before and after the GPR stimulation, bottom trace) did not evoke a plateau potential. B: when the slow GPR-evoked depolarization failed to trigger a plateau potential, a short depolarizing current pulse (which was ineffective before GPR stimulation) triggered a prolonged plateau potential. C: the plateau-inducing effect of GPR stimulation was noncholinergic and persisted after blocking nicotinic and muscarinic receptors with d-tubocurarine (0.1 mM) and atropine (0.1 mM), respectively. GPR stimulation still evoked a slow depolarization (top trace), and a plateau potential was evoked by current injection. D and E: local application by pressure ejection of 5-HT (5-HT puff; 1 mM in pipette) onto the neuropil mimics the plateau-inducing effects of GPR stimulation. The 5-HT puff evoked a slow depolarization in DG (top traces), and a brief 1-nA current pulse triggered a prolonged plateau potential, with high-frequency spike activity; a series of current pulses of the same and larger amplitudes failed to trigger a plateau before 5-HT. The plateau potential terminated spontaneously (D) or was terminated prematurely by a short hyperpolarizing current pulse (E). Different cells in A–C, and D and E.

Effects of different gastropyloric receptor (GPR) stimulation frequencies on bistable plateau properties in dorsal gastric (DG). A: intracellular recordings from DG (top traces) show that increasing the GPR stimulation frequency from 5 to 20 Hz caused the DG to fire a plateau potential at progressively shorter times after onset of the stimulation. Note the discrete nicotinic excitatory postsynaptic potentials, evoked by low-frequency stimulation in A. D: recordings from a different DG motor neuron show that the serotonergic plateau induction was present when a GPR cell was stimulated in trains from 2 to 20 Hz. Plateaus were evoked by brief depolarizing current pulses (bottom traces) and were more prolonged after 20-Hz GPR stimulation than 2-Hz stimulation.
pulses (Fig. 2E). In some cases the slow depolarization evoked by a 5-HT puff could by itself reach the threshold for triggering a plateau potential (Figs. 4A and 7, A and B). A brief application of 5-HT thus closely mimics the noncholinergic plateau-inducing effect of GPR activity on the DG motor neuron.

Synergistic action of ACh and 5-HT on plateau induction in DG

From the above experiments it thus appears that the two transmitters, ACh and 5-HT, which are co-released during GPR activity, act synergistically on the DG motor neuron: 5-HT induces the ability to generate plateau potentials, and ACh provides a rapid depolarization for triggering the plateau potential. When the nicotinic EPSPs are blocked, the time to onset of the plateau is delayed, or the plateau is not generated except when an external depolarization is provided (Fig. 2C).

In the absence of curare (i.e., with intact nicotinic EPSPs), the time to onset for the plateau is dependent on the frequency of the GPR stimulation (Fig. 3). When the frequency was increased from 5 to 20 Hz, with the number of spikes in the train kept constant, the plateau was triggered progressively earlier (Fig. 3A–C). As the frequency increases, there is an increased temporal summation of the nicotinic EPSPs (note the EPSPs in Fig. 3A on the rising phase of the depolarization). The co-release of ACH with 5-HT thus provides an additional depolarization to accelerate the rate at which GPR stimulation evokes a plateau potential.

Enough 5-HT is released from GPR terminals to induce plateau capabilities even at very low stimulus frequencies. When a GPR cell was stimulated at 2 Hz, it could not alone evoke a plateau potential. However, a brief depolarizing current injection, which was ineffective before GPR stimulation, could evoke a full plateau potential under these conditions (Fig. 3, D and E; n = 3).

In the rest of this paper and the following paper (Kiehn and Harris-Warrick 1992), we will focus only on the 5-HT-mediated and noncholinergic effects of GPR stimulation on the DG motor neuron.

**5-HT-mediated plateau induction is independent of the spike-generating mechanism**

The 5-HT-induced plateau potential in the DG motor neuron was independent of the spike-generating mechanism. When all spike activity was blocked with 0.1–1 μM TTX, a short depolarizing pulse (Fig. 4B; control response in normal medium depicted in Fig. 4A) could still trigger a plateau in DG. The slow 5-HT-evoked depolarization (\(\uparrow\)) preceding the plateau persisted in TTX and was often sufficiently large to trigger a partial plateau response on its own (Fig. 7, A and B). This TTX insensitivity allowed us to study the ionic mechanism for the plateau induction in more detail.

**Ionic mechanism of the plateau induction**

**5-HT EVOSES DUAL CONDUCTANCE CHANGES.** To get a hint on the ionic mechanisms underlying the 5-HT-induced plateau induction, we measured the relative changes in input resistance that accompany the 5-HT-evoked depolarization. In normal saline (plus TTX) at resting potential (−55 to −65 mV), the 5-HT evoked depolarization was accompanied by either no obvious changes (Fig. 5A) or a small increase (10–15%) in apparent input resistance, as measured by the size of the voltage deflection induced by short, low-amplitude hyperpolarizing current pulses. When one-half of the extracellular sodium was replaced with tris(hydroxymethyl)aminomethane (Tris; \(n = 3\)) or choline (\(n = 1\)), the apparent resting input resistance increased, and the 5-HT-evoked depolarization was now accompanied by a more pronounced increase in apparent input resistance (Fig. 5B). Conversely, when the extracellular calcium was reduced to 25% or completely replaced with magnesium, the 5-HT-evoked depolarization was accompanied by an apparent decrease in input resistance (\(n = 4\); Fig. 5C). DG also depolarized 7–15 mV when extracellular calcium was reduced (Fig. 7).

These results suggest that 5-HT evokes a slow depolarization and induces plateau properties in the DG motor neuron by a mixed conductance increase and decrease mechanism. We have studied the conductance changes produced by 5-HT more rigorously with two electrode voltage-clamp experiments.

**5-HT-INDUCED CONDUCTANCE CHANGES IN VOLTAGE CLAMP.** When DG was voltage clamped, a puff of 5-HT induced an inward current of prolonged duration, corresponding to the prolonged subthreshold depolarization seen under current-clamp conditions (Fig. 6A). The current versus voltage relationship of the 5-HT-induced current (measured as the peak current at different holding potentials) had an inverted I-shape and was inward over the entire voltage range studied (Fig. 6B). The minimum response was seen near the typical resting membrane potential (−55 mV) of the quiescent DG motor neuron.

Small hyperpolarizing voltage steps (−10 mV) were made before and during the 5-HT puff to monitor changes in input conductance. The plot of the relative changes in input conductance evoked by 5-HT (measured as the difference before and at the peak of the 5-HT–induced inward current) showed a complex voltage dependence. At the nor-
5-HT exerts its effect on the DG motor neuron by affecting multiple conductance, one of which is calcium sensitive.

**Combined effect of calcium substitution and extracellular cesium on the 5-HT-evoked plateau induction**

At the resting potential (−54 mV) in normal saline (plus TTX), DG responded to a puff of 5-HT with a slow depolar-
FIG. 7. Combined actions of Cs⁺ and low extracellular Ca²⁺ on the serotonin (5-HT) evoked slow depolarization and regenerative response under current clamp. All records from the same cell. A and A1: in normal saline [plus tetrodotoxin (TTX), 1 μM], 5-HT (1 mM) evoked a prolonged slow depolarization with a regenerative plateau-like depolarization at resting potential (−54 mV). When the cell was hyperpolarized by constant-current injection (A1; −58 mV), the regenerative response was abolished, leaving the slow depolarization. B and B1: adding 5 mM CsCl caused the cell to hyperpolarize to a new resting potential (B1; −58 mV), where the 5-HT-evoked depolarization was markedly reduced compared with the pre-Cs⁺ level (A1). When the cell was depolarized to the original resting potential of −54 mV (by constant-current injection), 5-HT still evoked a regenerative response. C and C2: reducing extracellular calcium to 25% (substituted with magnesium and still in the presence of 5 mM CsCl) caused the cell to depolarize to −46 mV (C2). At this new resting potential, 5-HT still evoked a slow depolarization but without a marked regenerative response. When the membrane potential was adjusted to −54 mV (C) or −58 mV (C1), the 5-HT-evoked depolarization was substantially reduced both compared with control (A and A1) and in 5 mM CsCl alone (B and B1).

Several conclusions can be drawn from this experiment. First, a calcium-dependent and a Cs⁺-sensitive conductance are active at rest, and they both contribute to the resting membrane potential, but in opposite directions (the calcium-dependent conductance causing a steady hyperpolarization and the Cs⁺-sensitive conductance causing a steady depolarization). Second, the 5-HT-evoked depolarization is mediated to a large degree (but perhaps not completely) by these two conductances. Third, 5-HT can still trigger a regenerative plateau-like response in 5 mM CsCl, although the rise time for the depolarization is slightly reduced. This was also observed with GPR-evoked plateaus (data not shown), indicating that the Cs⁺-sensitive conductance plays a role in the initiation of the plateau.

Conductance mechanism underlying GPR-evoked plateau induction

Although these experiments could not be repeated during GPR stimulation, a similar mechanism seems to be responsible for the GPR-evoked plateau induction. Figure 8 illustrates two different DG motor neurons in saline with 0.1 mM d-tubocurarine added to block the fast GPR-evoked nicotinic EPSPs in DG. The membrane potential in Fig. 8A was −55 mV, and small-amplitude (−0.3 nA) hyperpolar-
izing current pulses were used to measure the change in input resistance. The GPR train (10 Hz) evoked a depolarization and an apparent increase in input resistance \((n = 6)\). In Fig. 8B a different DG motor neuron was held at a more hyperpolarized membrane potential \((-68 \text{ mV})\). When large-amplitude hyperpolarizing current pulses \((1.6 \text{ nA})\) were applied, there was a decrease in apparent input resistance during the GPR train (5 Hz; Fig. 6B). Note also that the rebound depolarization that followed the large-amplitude hyperpolarizing current pulse was amplified during GPR stimulation \((n = 3)\).

These results suggest that exogenously applied 5-HT and GPR stimulation evokes the capability to generate plateau potentials in the DG motor neurons by affecting the same ionic conductances and that at least part of the release of the plateau is mediated by a mixed conductance increase and decrease mechanism.

DISCUSSION

In the present series of experiments, we have investigated the mechanism for plateau potential induction in a crustacean motor neuron, the dorsal gastric (DG) motor neuron, by a set of peripheral serotonergic/cholinergic stretch-activated sensory cells, the GPR cells. Recordings from DG motor neurons under current-clamp and voltage-clamp conditions show that 5-HT application and GPR stimulation induce plateau potential capability by a mixed conductance increase and decrease mechanism.

Serotonergic modulation of plateau potential properties

GPR cells contain ACh and 5-HT as cotransmitters, and GPR activity has strong excitatory effects on DG (Katz and Harris-Warrick 1989). A brief train of GPR stimuli elicits an initial slow membrane depolarization, which, when it exceeds a certain voltage threshold, triggers a prolonged plateau potential capped by high-frequency action potentials. The initial depolarization is partly caused by GPR-evoked nicotinic EPSPs, but, like the GPR-evoked plateau potential capability, a low-amplitude slow depolarization persists after blocking nicotinic and muscarinic receptors (Katz and Harris-Warrick 1989). In this paper we have demonstrated that local application of 5-HT mimics the slow depolarization and the plateau induction evoked by GPR stimulation in the presence of cholinergic antagonists. The close similarities between the DG responses after local application of 5-HT and the noncholinergic responses to GPR stimulation lead us to conclude that 5-HT released during GPR cell activity is indeed responsible for the GPR-evoked induction of plateau potential capabilities in DG. Thus it is not necessary to postulate the existence of additional transmitters in the GPR cells that should be responsible for the plateau induction, as previously suggested (Katz and Harris-Warrick 1989). The experiments also draw attention to the importance of the mode of exogenous transmitter application. Plateau potential induction was not observed in DG during bath application of 5-HT \((0.01-100 \mu\text{M}, n = 3)\) (O. Kiehn, unpublished observations), although 5-HT still caused DG to depolarize (Katz and Harris-Warrick 1989; O. Kiehn, unpublished observations). This difference in functional outcome caused by the two modes of transmitter application might be explained by several factors. First, 5-HT modulates more than one target conductance in DG (this paper; Kiehn and Harris-Warrick 1989). A brief train of GPR stimuli elicits a slow depolarization, which, when it persists after blocking nicotinic and muscarinic receptors (Katz and Harris-Warrick 1989). In this paper we have demonstrated that local application of 5-HT mimics the slow depolarization and the plateau induction evoked by GPR stimulation in the presence of cholinergic antagonists. Plateau potential induction was not observed in DG during bath application of 5-HT \((0.01-100 \mu\text{M}, n = 3)\) (O. Kiehn, unpublished observations), although 5-HT still caused DG to depolarize (Katz and Harris-Warrick 1989; O. Kiehn, unpublished observations). This difference in functional outcome caused by the two modes of transmitter application might be explained by several factors. First, 5-HT modulates more than one target conductance in DG (this paper; Kiehn and Harris-Warrick 1992), and a continuous stimulation of 5-HT receptors with a constant transmitter concentration (during bath application) will activate those membrane conductances with a very different time course than is achieved during local application of 5-HT or GPR stimulation. Second, during prolonged bath application, 5-HT might gain access to 5-HT receptors that are normally not accessible to the 5-HT released during GPR activity. This is especially relevant for stomatogastric neurons, because the ganglion is situated within the anterior ophthalmic artery and has access to blood-borne hormones, including 5-HT (Cooke and Sullivan 1982). It is interesting that a similar phenomenon recently has been observed in cultured neurons from the mollusk *Lymnaea stagnalis* (Syed et al. 1990). In this preparation continuous bath application of dopamine evoked a hyperpolarization in respiratory target neurons, whereas pulsatile application induced bursting, similar to the one induced by brief activation of a dopaminergic modulatory neuron.
In many species, plateau potentials and a bistable firing pattern are latent properties of motor neurons expressed only in the presence of exogenously applied neurotransmitters, such as excitatory amino acids (Engberg et al. 1984; Grillner and Wallén 1985), 5-HT (Hounsgaard et al. 1988; Hounsgaard and Kiehn 1983, 1989), noradrenaline (Conway et al. 1988), or muscarinic cholinergic agonists (Chrachi and Clarac 1990; Nagy et al. 1985). Transmitter regulation of active membrane properties of motor neurons therefore appears to play an important role in motor control. By identifying the transmitter responsible for the GPR-evoked plateau induction in DG, we have created a direct link between synaptic and exogenous transmitter regulation of these properties.

**GPR cells use multiple transmitters to modulate DG response pattern**

ACh and 5-HT act synergistically on the DG motor neuron. 5-HT endows DG with the ability to generate plateau potentials, whereas ACh provides a rapid depolarization that superimposed on the slow 5-HT-evoked depolarization carries the cell above threshold for triggering a plateau potential. In a semi-intact foregut preparation, the GPR cells are endogenously rhythmically active, firing bursts of action potentials starting at low frequencies (1-2 Hz) and with peak frequencies of 8-20 Hz (Katz et al. 1989). Over this frequency range, GPR cells can evoke plateau potential capability in DG. However, the slow depolarization evoked by 5-HT release alone is usually insufficient to trigger the plateau, and the synergistic interaction between ACh and 5-HT is therefore essential to secure a rapid onset of the plateau potential.

It is interesting that the ability of the GPR cells to induce plateau potentials in DG persists even at very low stimulus frequencies (2 Hz; Fig. 3). This suggests that 5-HT and ACh are co-released over the entire frequency range. A different result is seen in other systems, in which small transmitters are colocalized with peptides. Here the extent of cotransmission varies with stimulation frequency: small transmitters are released both at low and high frequencies, whereas peptides are released only on stimulation at higher frequencies (Bartfai et al. 1988, Whinn and Lloyd 1989).

**Mechanism of plateau induction**

In other systems, the generation of plateau potentials arises from activation of slowly or noninactivating Na⁺ conductances (Jahnsen 1986; Llinás and Sugimori 1980a; Smith et al. 1975; Staafström et al. 1982), Ca²⁺ conductances (Arbas and Calabresi 1987; Eckert and Lux 1976; Hounsgaard and Kiehn 1985, 1989; Jahnsen 1986; Llinás and Sugimori 1980b; Misgeld et al. 1986), or from some combination of these conductances. In accordance with previous observations in the STG (Gola and Silverston 1981; Harris-Warrick and Hamm 1987; Russell and Hartline 1984), we have shown that the 5-HT-evoked DG plateau potential is not dependent on the spike-generating mechanism. This indicates that the plateau itself is carried by TTX-insensitive sodium and/or calcium currents. In several experiments the plateau potential was blocked by short exposure of the DG to low extracellular calcium (Fig. 7), suggesting a strong calcium-dependent component in the plateau current. However, in some cells a small plateau response persisted in the presence of either low calcium or 10-20 mM cobalt, which suggests an additional Na⁺ component to the plateau. Because prolonged exposure to Na⁺-free saline, especially in combination with low calcium, caused irreversible damage to the DG motor neuron (Gola and Silverston 1981; Harris-Warrick and Flamm 1987; O. Kiehn, unpublished observations), we did not determine the precise nature of the plateau current itself.

The induction of plateau potentials can be caused by enhancement of inward and/or reduction of tonically active subthreshold outward current mechanisms. Thus both pharmacological enhancement of calcium currents and antagonists of K⁺ conductances have been shown to induce or enhance plateau responses in a variety of different neurons, including invertebrate motor neurons and vertebrate motor neurons (Gola and Silverston 1981; Hounsgaard and Kiehn 1985, 1989; Hounsgaard and Mintz 1988; Mosfeldt Laursen and Rekling 1989; Nagy et al. 1985; Russell and Hartline 1984; Schwindt and Crill 1980a,b; Tazaki and Cooke 1979), rat neostriatal neurons (Cherubini and Länfumey 1987; Misgeld et al. 1986), neurosecretory neurons (Bourque et al. 1986; Legendre et al. 1988), rat hippocampal gyrus dentatus neurons (Godfraind 1985), cerebellar Purkinje cells (Llinás and Sugimori 1980a), and goldfish solitary horizontal cells (Tachibana 1981). Consistent with these results, we show here that 5-HT released by GPR stimulation evokes a mixed conductance increase and decrease mechanism, which is at least partly responsible for the release of the plateau in the DG motor neuron. The conductance decrease evoked at voltages depolarized from rest in normal saline indicates that part of the 5-HT effect is mediated by a slow outward current mechanism, which is at least partly responsible for the release of the plateau. In saline with reduced calcium, 5-HT evoked a Cs⁺-sensitive depolarization (Fig. 7) and a conductance increase (Fig. 5C). This conductance increase was enhanced in normal saline at hyperpolarized voltages (Fig. 6). It therefore shares similarities with a hyperpolarization-activated inward current ($I_h$), found in many cells (Angstadt and Calabresi 1989; Halliwell and Adams 1982; Mayer and Westbrook 1983; McCormick and Pape 1990; Yanagihara and Irisawa 1980). An enhancement of this current by 5-HT would explain the conductance increase at hyperpolarized levels. Low concentrations of extracellular Cs⁺ are known to block $I_h$. We thus suggest that the 5-HT-induced mixed conductance change is mediated by a decrease of a calcium-dependent outward conductance and an increase of a (Cs⁺-sensitive) $I_h$. We investigate these possibilities further in our accompanying paper (Kiehn and Harris-Warrick 1997). This mechanism elicits an inward current with no reversal potential over a broad voltage range, which can explain the slow depolarization (a reflection of the induction of plateau potential capability) preceding the plateau potential. Because reduction of extracellular calcium causes the DG cell to depolarize, whereas adding low concentrations of CsCl to the extracellular saline cause the cell...
to hyperpolarize, we propose that both conductances are partially active at rest and contribute to the resting membrane potential. It is thus possible that serotonin's reduction of a calcium-dependent outward conductance and enhancement of a Cs+-sensitive inward conductance causes a shift in the balance between total inward and outward cell currents that is sufficient to permit activation of a persistent plateau state. Consistent with this view, previous findings suggested that muscarinic reduction of a subthreshold K⁺ conductance in stomatogastric neurons (Nagy et al. 1983) and serotonergic reduction of a slow afterhyperpolarization conductance in turtle motoneurons (Hounsgaard and Kiehn 1989) uncover a voltage-dependent Ca²⁺-mediated plateau conductance. Obviously, this does not exclude the possibility that 5-HT has additional effects on DG conductances. We will consider this further in the following paper (Kiehn and Harris-Warrick 1992). Finally, although it was not possible to perform ideal experiments with GPR stimulation, it appears that 5-HT released during GPR stimulation can also excite DG by a conductance increase and decrease mechanism.

The present study has provided a first description of a unique mechanism for motor neuron plateau potential induction by transmitter application and stimulation of an identified neuromodulatory neuron. These findings provide insights into how synaptically released transmitter can modulate intrinsic membrane properties in the context of motor behavior and have implications for the mechanism of transmitter regulation of plateau properties in both interneuronal and vertebrate nervous systems.

We thank our colleagues for critical comments on an earlier version of this manuscript. We thank Dr. Paul Katz for providing the recordings in Fig. 2, A–C, and for many helpful discussions of the data. The research was supported by National Institute of Neurological Disorders and Stroke Grants NS-17322 and NS-23501, United States Department of Agriculture Hatch Act Grant NYC 191410 (to R. Harris-Warrick), and the Danish Medical Research Council (to O. Kiehn).

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Received 22 October 1991, accepted in final form 24 March 1992.

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