PGE_2 hyperpolarizes gallbladder neurons and inhibits synaptic potentials in gallbladder ganglia

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Jennings, Lee J., and Gary M. Mawe. PGE_2 hyperpolarizes gallbladder neurons and inhibits synaptic potentials in gallbladder ganglia. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G493–G502, 1998.—Gallbladder prostaglandin E_2 (PGE_2) levels are significantly elevated in pathophysiologic conditions, resulting in changes in gallbladder motility or secretion that may involve actions of the prostanoi in intramural ganglia. This study was undertaken to examine the effects of PGE_2 on neurons of the intramural ganglia of the guinea pig gallbladder. Application of PGE_2 by microejection or superfusion elicited a complex triphasic change in the resting membrane potential (RMP). For example, application of PGE_2 by microejection (100 µM) resulted in a brief hyperpolarization (mean duration 11.1 ± 1.3 s), followed by a midphase repolarization toward or above RMP (mean duration 50.7 ± 8.1 s), and finally a long-lasting hyperpolarization (mean duration 157.3 ± 36.7 s). Associated with these PGE_2-evoked alterations in RMP were changes in input resistance measured via injection of hyperpolarizing current pulses. An examination of the action potential afterhyperpolarization (AHP) during the PGE_2-evoked response revealed an attenuation of both the amplitude and duration of the AHP. However, only a slight increase in excitability of gallbladder neurons in the presence of PGE_2 was evident in response to depolarizing current pulses, and PGE_2 did not cause the cells to fire spontaneous action potentials. Application of PGE_2 reduced the amplitudes of both fast and slow excitatory synaptic potentials. These results suggest that increased prostaglandin production may decrease ganglionic output and therefore contribute to gallbladder stasis.

METHODS

The methods that were employed in this study have been described previously in considerable detail (21, 23). Adult guinea pigs of either sex, weighing 250–350 g, were used for this study. Animals were anesthetized with isoflurane until no hindlimb reflex was evident and then exsanguinated. This method was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Vermont. The gallbladder was immediately removed and opened with a single incision from the end of the cystic duct to the base of the gallbladder. It was then pinned flat, mucosal side up, under recirculating ice-cold Krebs solution in a dish lined with Sylgard 184 elastomer (Dow Corning, Midland, MI). The mucosal layer and underlying connective tissue were gently removed with forceps under microscopic observation. The preparations were then pinned out in a Sylgard-lined tissue chamber and placed on the stage of an inverted microscope (Diaphot, Nikon). Individual ganglia were visualized at ×200 with Hoffman modulation contrast optics (Modulation Optics, Greenvite, NY).

The preparations were continuously perfused at a rate of 10–12 ml/min with a modified Krebs solution that was aerated with 95% O_2-5% CO_2, and the temperature was 36–37°C at the recording site. The solution contained (in mM) 121 NaCl, 5.9 KCl, 2.5 CaCl_2, 1.2 MgCl_2, 25 NaHCO_3, 1.2 NaH_2PO_4, and 8 glucose. Nifedipine (0.5 µM) was added to the Krebs solution to minimize smooth muscle contractions.

Glass microelectrodes used for intracellular recording were filled with 2.0 M potassium chloride and had resistances in

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the range of 50–110 MΩ. A negative-capacity compensation amplifier (Axoclamp 2A; Axon Instruments, Foster City, CA) with bridge circuitry for injecting positive and negative current pulses (0.1–0.5 nA, 0.5–500 ms, 0.5–1 Hz) was used to record membrane potentials. Synaptic inputs were elicited using monopolar extracellular electrodes made from Teflon-insulated platinum wire (25-µm diameter) to apply 0.2- to 0.5-ms duration stimuli (0.2–20 Hz frequency) to the interganglionic connectives.

In some experiments, the composition of the Krebs solution was altered to facilitate the examination of a particular response. In these instances, molarity was compensated for by adjustment of NaCl within the solution. For example, when the K+ concentration was raised from 5.9 to 20 mM, the NaCl concentration was reduced from 121 to 107 mM.

Compounds were applied by pressure microjection from glass micropipettes (0.01–1.0 mM in Krebs solution; 15- to 20-µm tip diameter) by pulses of nitrogen gas (300 kg/cm²; 10–1,000 ms in duration) or by addition to the circulating Krebs solution. The distance between the tip of the spritz micropipette and the impaled neuron was maintained between 50 and 100 µm. PGE2 was purchased from Sigma Chemical (St. Louis, MO). For stock solutions, PGE2 was dissolved in dimethyl sulfoxide (DMSO) and diluted at least 1,000 times before tissue application. Microjection or superfusion of the drug vehicle (DMSO) at relevant concentrations did not have any measurable effect on the active or passive properties of gallbladder neurons.

Averaged numerical values are presented as means ± SE, and P < 0.05 (Student’s t-test) was considered significant.

RESULTS

Data were obtained from 187 gallbladder neurons in 141 different guinea pig gallbladders. The passive and active membrane characteristics of these cells were similar to those reported previously for guinea pig gallbladder neurons (21). The cells usually generated only one action potential at the onset of a depolarizing current pulse, regardless of the duration or amplitude of the pulse, and anodal break action potentials were rarely observed in control conditions. The cells studied here had a mean resting membrane potential (RMP) of −52.3 ± 2.1 mV and a membrane input resistance of 75.8 ± 6.2 MΩ.

PGE2 elicits a triphasic change of the RMP. Application of PGE2 to gallbladder neurons by either microjection or superfusion elicited a complex triphasic change of the RMP. The characteristic PGE2-evoked response was triphasic, with three distinct components: early, mid-, and late phases.

The early phase consisted of a membrane hyperpolarization whose magnitude was measured from the RMP to the peak of the hyperpolarization. The mid-phase of the PGE2-evoked response was characterized by a membrane repolarization back toward the initial RMP, which in the majority of neurons led to a membrane depolarization. The amplitude of this mid-phase was calculated from the peak of the preceding membrane hyperpolarization to the peak of the repolarization/depolarization. The late phase of the response consisted of a second membrane hyperpolarization whose amplitude was measured from RMP to the peak of the hyperpolarization.

The duration of the early phase hyperpolarization was calculated by measuring the time from the onset of the response to the point that the cell repolarized to the RMP. In cells that did not return to the RMP, the slope of the repolarization was extrapolated to the RMP and this provided the second time point. The duration of the mid-phase repolarization was taken from the point that the cell returned to the RMP (or from slope extrapolation) after the initial hyperpolarization to the point at which the late phase hyperpolarization started. The duration of the late phase hyperpolarization was measured from the end of the preceding repolarization/depolarization to a return to the RMP or until the impalement was lost. It was impossible to separate these phases and examine them individually, and as a consequence it is likely that each phase could have had an effect on the amplitude and/or the duration of the preceding phase.

Microjection of PGE2. Application of PGE2 by pressure microjection (1 µM–1 mM; 0.1–3 s) elicited a complex change of the RMP of gallbladder neurons as mentioned above (Fig. 1A). All three phases were seen at all concentrations applied. For example, at a concentration of 100 µM (n = 12), the early phase consisted of membrane hyperpolarization having a mean magnitude of 9.3 ± 1.6 mV (range: 3.4–18.9 mV) and a mean duration of 11.1 ± 1.3 s (range: 5.4–21.4 s). A decrease in membrane input resistance (16.6 ± 7.8%), measured by changes in response to injection of hyperpolarizing current pulses, was associated with this early hyperpolarization.

The mean peak amplitude of the mid-phase repolarization (at 100 µM) was 14.2 ± 1.3 mV (range: 5.4–21.4 mV) with a mean duration of 50.7 ± 8.1 s (range: 17.8–100 s). A decrease in membrane input resistance (58.6 ± 4.7%) was evident during the mid-phase membrane repolarization.

The late phase of the PGE2-induced triphasic response consisted of a long-lasting hyperpolarization with a mean peak amplitude of 4.3 ± 0.7 mV (range: 1.2–8.7 mV). Of the cells exposed to 100 µM PGE2 by microjection only 5 of the 12 returned to the RMP. In these cells, the mean duration of the late phase was 157.3 ± 36.7 s (range: 92.9–220 s). An increase in membrane input resistance (18.5 ± 7.4%) accompanied the late phase hyperpolarization.

The possibility that any component of the PGE2-induced potential change was a secondary effect due to PGE2-induced release of neurotransmitters from nerve terminals was tested by application of PGE2 in the presence and absence of tetrodotoxin (TTX). In three cells, the presence of 0.5 µM TTX (Fig. 1B) did not significantly alter the early phase amplitude or duration (P = 0.21 and 0.18, respectively), the mid-phase amplitude or duration (P = 0.34 and 0.29, respectively), or the late phase amplitude or duration (P = 0.48 and 0.51, respectively). In addition, we examined the response of gallbladder neurons to PGE2 (1 mM, 400 ms) in a Ca²⁺-free Krebs solution (3.7 mM MgCl) and compared these with control responses. On examination (n = 3), neither the early phase amplitude or...
duration ($P = 0.51$ and 0.43, respectively), the mid-phase amplitude or duration ($P = 0.14$ and 0.8, respectively), nor the late phase amplitude or duration ($P = 0.18$ and 0.47, respectively) was significantly altered compared with control responses.

The magnitude of the response to PGE$_2$ was dependent on the duration of PGE$_2$ microejection. The minimal pulse duration that resulted in a detectable response varied among the cells, but was usually in the range of 50 to 100 ms. Progressive increase in duration of PGE$_2$ application resulted in slightly larger responses, with a maximal response usually occurring with application durations in the range of 1–3 s. The maximal response in a given cell was quite reproducible when the preparation was rinsed for periods of 5 min between applications.

Superfusion of PGE$_2$. Superfusion of PGE$_2$ (10 pM-10 µM) evoked a concentration-dependent triphasic change in resting membrane potential (RMP) with characteristics similar to those seen when PGE$_2$ was applied by microejection (Fig. 2). The peak amplitude (11.3 ± 1.9 mV; range: 6.1–19.2 mV) of the early hyperpolarization was seen at a concentration of 1 µM. Of the eight cells studied at this concentration, two cells were lost immediately after the initial hyperpolarization, four cells had a mean duration of 22.1 ± 3.9 s (range: 13.9–25.5 s), and two cells remained hyperpolarized for longer than 4 min. The maximum amplitude of the mid-phase repolarization (6.8 ± 0.7 mV; range: 2.5–9.5 mV) occurred at 10 µM and had a duration of 62.5 ± 11.5 s (range: 22.3–112.1 s; $n = 6$). The amplitude of the late phase hyperpolarization also was greatest at a concentration of 10 µM (8.6 ± 1.9 mV; range: 3.3–16.1 mV), and of the six cells studied only two returned to the RMP (duration >2 min). The other cells remained hyperpolarized throughout the recording. Superfusion of the drug vehicle (DMSO) at relevant concentrations did not have any measurable effect on the membrane potential of gallbladder neurons.

Ionic currents that underlie the PGE$_2$-evoked responses. To examine the ionic currents potentially involved in the complex PGE$_2$-evoked voltage response, PGE$_2$ was applied to cells that were current clamped at potentials more positive or more negative than the RMP (Fig. 3).

In eight cells, the amplitude of the early phase hyperpolarization decreased at more negative potentials and also decreased when the RMP was clamped toward 0 mV. Because of the rectification behavior of these neurons, as mentioned above, it was difficult to depolarize the...
neurons to levels that were sufficiently positive to detect a true reversal of the mid-phase response. However, estimated reversal potentials were obtained by plotting the membrane potentials against the responses obtained and using a line of best fit (assuming linearity) to derive the estimated reversal potentials. The mean estimated reversal potential for the mid-phase of the PGE$_2$-evoked response was $-3.2 \pm 11.6$ mV.

The amplitude of the late phase hyperpolarization became larger at more negative membrane potentials, but the response could often be reversed when the cells were current clamped at or close to 0 mV. The mean estimated reversal potential for this late phase hyperpolarization was $-30.9 \pm 7.8$ mV.

Given the reversal potential data and decrease in input resistance, we hypothesize that the PGE$_2$-evoked early hyperpolarization may involve the activation of a K$^+$ conductance. To test this, reversal potential measurements were made using Krebs solution containing either 5.9, 12, or 20 mM KCl. When the KCl concentration was increased from 5.9 to 12 mM the mean reversal potential was shifted from $-81.9 \pm 11.6$ to $-77.8 \pm 4.3$ mV ($n = 3$). The predicted K$^+$ equilibrium with 12 mM KCl equaled $-73.8$ mV. In Krebs solution with 20 mM KCl the mean reversal potential was estimated to be $-68.2 \pm 6.5$ (n = 5), whereas the predicted K$^+$ equilibrium potential was $-60.2$ mV (Fig. 4). In addition, the amplitude of the early phase of hyperpolarization was decreased as the KCl concentration was increased. Also, a true reversal of the hyperpolarization could be elicited at the higher KCl concentrations. The mid- and late phases of the PGE$_2$-evoked potential change were not significantly altered by the presence of the higher KCl concentrations. The reversal potential estimated for the mid-phase of the PGE$_2$ response was $-9.9 \pm 1.7$ mV ($n = 3$) in 12 mM KCl and was $-8.0 \pm 4.7$ mV ($n = 4$) in 20 mM KCl. The mean estimated reversal potential for the late phase was $-22.4 \pm 2.2$ mV ($n = 3$) in 12 mM KCl and was $-25.1 \pm 7.2$ mV ($n = 4$) in 20 mM KCl.

To test further the hypothesis that the early hyperpolarization phase was due to the activation of a K$^+$ conductance, we determined the effect of barium chloride (BaCl$_2$), a nonspecific blocker of K$^+$ channels (32), on the PGE$_2$ response. Barium chloride, at a concentration of 2 mM, was applied to the whole mount preparation ($n = 8$). PGE$_2$ was then applied (100 µM, 200 ms), and the PGE$_2$ response in BaCl$_2$ was compared with control responses. BaCl$_2$ depolarized the gallbladder neurons (mean, $11.1 \pm 2.7$ mV; range: 3.9–29.1 mV). In addition, BaCl$_2$ increased excitability of neurons as demonstrated by the development of anodal break excitation at the offset of a hyperpolarizing current pulse (Fig. 5). To compare the PGE$_2$ response in BaCl$_2$ with that obtained in control conditions, the neurons were current clamped back to the RMP to negate the BaCl$_2$-induced depolarization. In the presence of BaCl$_2$, the mean early phase hyperpolarization elicited by PGE$_2$ was decreased by $-50\%$ (control, 5.0 ± 1.6 mV; range: $-12.3$ to $-1.2$ mV; BaCl$_2$, $2.7 \pm 0.9$ mV; range: $-6.2$ to 0 mV; P < 0.05; Fig. 5). The mid- and late phases of the PGE$_2$-evoked response were not significantly affected by the presence of BaCl$_2$.

PGE$_2$ attenuates action potential afterhyperpolarization. The action potential afterhyperpolarization (AHP) of gallbladder neurons typically has a magnitude of

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**Fig. 2.** Application of PGE$_2$ elicited a concentration-dependent (10 pM–10 µM) triphasic change in RMP of gallbladder neurons consisting of an early hyperpolarization phase, followed by a mid-phase repolarization toward or above RMP, and finally a long-lasting hyperpolarization phase. Maximal responses were seen at a concentration of 1 µM for the early phase and at 10 µM for the mid- and late phases. Nos. in parentheses indicate no. of cells studied at each concentration, and the RMP (indicated by the dashed line) of the cell shown here was $-48$ mV.
15 mV and a duration of ~170 ms (21). Previous work from our laboratory (21, 26) has demonstrated that the AHP involves at least two Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductances, an early conductance that is tetraethylammonium sensitive and a later conductance that is apamin sensitive. In this study, we tested whether PGE\textsubscript{2} influenced AHP parameters. To analyze the AHP, single action potentials were generated using stimulation pulse width durations that terminated on the upstroke of the action potential (4–7 ms). In 15 cells, AHP control parameters were similar to those previously reported. Measurements were also taken of the magnitude of the AHP at a point 80 ms from the start of the AHP to detect any changes in the apamin-sensitive K\textsuperscript{+} conductance.

Application of PGE\textsubscript{2} by either superfusion or picospritz reduced all parameters of the AHP that were studied (Fig. 6). The magnitude of the AHP was reduced by 30% (control, 15.6 ± 0.8 mV; PGE\textsubscript{2}, 10.9 ± 1.4 mV; \(P < 0.001\)), the duration was reduced by 27.6% (control, 209.1 ± 11.0 ms; PGE\textsubscript{2}, 151.3 ± 17.9 ms; \(P < 0.003\)), and the amplitude of the AHP measured at 80 ms was reduced by 42.3% during PGE\textsubscript{2} application (control, 7.8 ± 1.0 mV; PGE\textsubscript{2}, 4.5 ± 1.1 ms; \(P < 0.01\)). In 8 of the 15 cells, the parameters of the AHP fully recovered to their control values after washout of the PGE\textsubscript{2} application.

When the gallbladder neuron AHP is attenuated by apamin (20) or substance P (23), an increase in excitability has been detected as an increase in the number of spikes generated during a depolarizing current pulse. As noted above, the neurons of the guinea pig gallbladder are relatively inexcitable under normal conditions; they typically generate only one or two action potentials at the onset of a prolonged depolarizing current pulse, and they rarely exhibit anodal break activity. To
determine whether PGE₂ altered the excitability of gallbladder neurons, action potential generation was evaluated before and after application of PGE₂. During these experiments, the cells were maintained electrotonically at their RMP during the late phase hyperpolarization of the PGE₂ response to negate any change in driving forces.

Only a minimal increase in excitability was detected in gallbladder neurons on application of PGE₂ by either superfusion or microejection. Application of PGE₂ did not initiate spontaneous action potential generation or result in anodal break activity at the offset of a hyperpolarizing current pulse. In 7 of 13 cells tested, an increase in the number of action potentials during a depolarizing current pulse was noted. However, the increase in action potential activity that was noted was slight compared with increased excitability in response to apamin or substance P.

PGE₂ inhibits fast synaptic transmission. All neurons in guinea pig gallbladder ganglia exhibit nicotinic fast EPSPs in response to stimulation of interganglionic fiber bundles (21). The vagus nerves are a major source of these fast excitatory synaptic inputs to gallbladder neurons (25). Experiments were conducted to determine whether PGE₂ affected fast EPSPs in gallbladder ganglia. Fast synaptic events were elicited by stimulating interganglionic nerve bundles (0.5 Hz, 0.5 ms, 1–10 V) before and during PGE₂ application. Data were obtained by measuring fast EPSP amplitudes from signal averages of five consecutive events. PGE₂...
caused a 67% reduction in the fast EPSP amplitude (control, 8.1 ± 3.4 mV; range: 4.7–13.4 mV; PGE2, 2.7 ± 3.6 mV; range: 0–8.7 mV; P < 0.05; Fig. 7). In three of the six cells, PGE2 reversibly eliminated the fast EPSP.

To determine whether PGE2 was acting presynaptically to suppress the fast EPSP, the effect of PGE2 on the responsiveness of neurons to ACh (0.1 mM, 100-ms microejection) was tested. If PGE2 was acting postsynaptically, we would expect a reduction of the ACh response after PGE2 application. The responsiveness of gallbladder neurons to ACh was not significantly altered by PGE2 (control, 9.2 ± 1.5 mV; PGE2, 8.7 ± 2.9 mV; n = 4).

PGE2 attenuates slow synaptic transmission. Slow EPSPs can be elicited in ~40% of gallbladder neurons (21). These long, slow depolarizations can be blocked by omission of Ca2+ in the superfusing solution, but not by atropine. Results obtained in previous studies (9, 19, 24) suggest that the mediators of this response include substance P and calcitonin gene-related peptide. Because changes in both prostaglandin levels and sensory afferent fiber plasticity have been implicated in inflammation, we examined the effects of PGE2 on slow EPSPs. Slow EPSPs were elicited in eight cells by stimulating interganglionic nerve bundles supramaximally at a high rate (20 Hz, 5-s train, 0.5-ms pulse width). Electrical stimulation was repeated during the PGE2-induced late phase hyperpolarization, with the cell current clamped to the RMP. Because the slow EPSP is subject to desensitization, sEPSPs were elicited in 15-min intervals. PGE2 reduced the amplitude of the slow EPSP by 42% (control, 5.2 ± 1.5 mV; range, 2.8–7.5 mV; PGE2, 3.0 ± 1.1 mV; range: 0–9.2 mV; P < 0.05; Fig. 8). After a 30-min washout period the magnitude of the slow EPSP was still reduced (27.3%) compared with control.

**DISCUSSION**

The aim of this study was to investigate the effects of PGE2 on neurons within the wall of the guinea pig gallbladder. The data presented here demonstrate that PGE2 acts directly on gallbladder neurons to elicit a complex triphasic change in the RMP and a decrease in the duration and amplitude of the AHP. Each component of the triphasic response was concentration dependent, associated with a change in input resistance, and changed in amplitude when the membrane potential was electrotonically increased or decreased. In addition to the direct effects of PGE2 on gallbladder neurons, PGE2 acted presynaptically to attenuate both fast and slow excitatory synaptic responses.

The actions of PGE2 have been studied directly in the myenteric ganglia and indirectly in the submucosal ganglia preparation of the guinea pig bowel (5, 6, 10). In myenteric neurons, PGE2 causes a monophasic, long-lasting depolarization and the activation of action potentials in myenteric type 1/S cells. In the myenteric plexus preparation, no hyperpolarizing responses were reported and the mechanism of the depolarizing response was not investigated. The effect of PGE2 on the membrane potential of gallbladder neurons is relatively complex, consisting of an early hyperpolarization, a mid-phase repolarization, and a long-lasting late hyperpolarization. The application of PGE2 did not induce action potential generation in gallbladder neu-
rons. A response to PGE\textsubscript{2}, reminiscent of that observed in gallbladder neurons, has been observed in canine renal epithelioid cells, which undergo a similar triphasic change in membrane potential in the presence of either PGE\textsubscript{2} or arachidonic acid (33). Steidl et al. (33) suggest that the electrical changes observed in their cells were the result of an increased K\textsuperscript{+} conductance followed by an increase in Cl\textsuperscript{-} conductance and that a return to the hyperpolarization state was due to a decrease in the chloride conductance.

Several lines of evidence suggest that the early hyperpolarization detected in gallbladder neurons involves the activation of a K\textsuperscript{+} conductance. This component of the response is characterized by decreased input resistance, a reversal potential that correlates with E\textsubscript{K}, and sensitivity to Ba\textsuperscript{2+}. The conductances responsible for the mid-phase repolarization and the late phase hyperpolarization are not resolved, but they probably are not due to movement of K\textsuperscript{+}. The reversal potentials of the mid- and late phases of the PGE\textsubscript{2} response in gallbladder neurons did not correlate with predicted E\textsubscript{K} in normal or elevated K\textsuperscript{+} conditions. The mid-phase repolarization may involve the activation of a nonselective cation conductance, because it is associated with a decrease in input resistance and its reversal potential is near 0 mV. From the data obtained, it is difficult to make any assumptions concerning the identity of the ionic current(s) involved in the late phase hyperpolarization except to suggest that it probably does not involve the movement of K\textsuperscript{+}.

Results reported here, related to changes in the AHP and evoked synaptic potentials, indicate that Ca\textsuperscript{2+}- and Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductances may be suppressed by PGE\textsubscript{2} in gallbladder ganglia. The AHP of gallbladder neurons is composed of two consecutive Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductances that are sensitive to tetraethylammonium and apamin, respectively (21). The amplitude of the AHP is dependent on the first conductance, and the duration is related to the second conductance. In the presence of PGE\textsubscript{2}, the AHP of gallbladder neurons was reduced in amplitude and duration, indicating that both K\textsuperscript{+} conductances were reduced either indirectly or directly by PGE\textsubscript{2}. Attenuation of the AHP by prostaglandins has also been observed in enteric neurons of the guinea pig submucosal and myenteric plexi (5, 8) as well as in rabbit visceral afferent neurons (7, 38). In rabbit sensory neurons, the AHP was inhibited by PGE\textsubscript{2} in the presence of a Ca\textsuperscript{2+} ionophore, indicating that a reduction in Ca\textsuperscript{2+} influx was not responsible for the PGE\textsubscript{2}-induced inhibition of the AHP.

In guinea pig gallbladder ganglia, vagal efferent fibers provide the principal source of nicotinic fast synaptic input (25). Because PGE\textsubscript{2} reduces the amplitude of EPSPs but does not alter the responsiveness of gallbladder neurons to exogenously applied ACh, PGE\textsubscript{2} is likely to attenuate the release of ACh from preganglionic vagal terminals. This is a significant observation, because it provides another example of the importance of vagal efferent terminals as a target of modulatory inputs in this system. Other examples include CCK, which acts at physiological concentrations on vagal terminals to increase ACh release, and activation of sympathetic nerves, which decreases ACh release from vagal terminals (21, 22).

The slow EPSP observed in the gallbladder is mediated primarily by substance P via an NK\textsubscript{3} receptor (24), although calcitonin gene-related peptide may provide a minor component of the response (9). We have previously hypothesized that the sEPSP may be a local axonal reflex response and could act to increase ganglionic output in an effort to increase gallbladder luminal pressure and expel any obstructions (9). The data presented here show that PGE\textsubscript{2} significantly decreases the amplitude of the sEPSP. In a similar study in the myenteric plexus of the guinea pig, PGE\textsubscript{2} did not decrease the amplitude of sEPSPs (5), but in the submucosal plexus, the related prostanoid PGD\textsubscript{2} did decrease the amplitude of these events (8).

Prostaglandins have been shown to inhibit transmitter release in several systems. For example, release of norepinephrine is attenuated by PGE\textsubscript{2} in the colon (41), in the heart (20), and at the iris-ciliary body (30), and PGE\textsubscript{2} decreases nonadrenergic, noncholinergic transmitter release in the rabbit stomach (1). In the gut, PGE\textsubscript{2} decreases ACh release (26, 34) and suppresses fast EPSPs in myenteric ganglia (5). In some cases, a prostaglandin induced an increase in ACh output in the presence of PGE\textsubscript{2} (4, 11, 15), but this may be due to a direct action of PGE\textsubscript{2} on enteric neurons. It is possible that, in the gallbladder and other systems, prostaglandins act through an inhibitory effect on Ca\textsuperscript{2+} channels to decrease transmitter release. In rat sympathetic ganglia, PGE\textsubscript{2} has been shown to cause a rapid reduction of the Ca\textsuperscript{2+} current, which is due to a depolarizing shift in the activation of N-type Ca\textsuperscript{2+} channels (12).

When considering the overall actions of prostaglandins in the biliary tract, a pertinent consideration is that treatment with cyclooxygenase inhibitors relieves biliary tract pain (35, 36). As prostaglandins are known to contract gallbladder muscle, this might suggest that a reduction in prostaglandin production may diminish gallbladder contractility, hence creating a decrease in gallbladder luminal pressure and leading to biliary pain relief. However, it is more likely that the reduction in prostaglandin production acts to prevent an increase in the sensitivity of primary afferent nerves within the organ. Prostaglandins have the ability to increase the sensitivity of primary afferents to noxious stimulation (31, 42). A subset of the sensory nerve fibers that innervate the gallbladder are selectively sensitive to noxious stimulation, indicating that pure visceral pain afferent fibers exist in the gallbladder (2). Taken together, these observations indicate that a decrease in prostaglandin production by cyclooxygenase inhibitors may prevent sensitization of the primary afferent fibers and lead to biliary pain relief despite any direct effects of prostaglandins on muscle or ganglionic output. In support of this hypothesis, it has been observed that in human tissue (17, 39) as well as in some animal models (3), gallbladder muscle becomes desensitized to prostaglandins. With the contractile effect of prostaglandin...
diminished with time, chronic prostaglandin production may contribute to the gallbladder stasis by effectively denervating the tissue. Long-lasting hyperpolarization of the neurons and suppression of fast and slow excitatory input would significantly decrease ganglionic output since gallbladder neurons need to be synchronically driven to generate action potentials.

In conclusion, PGE2 elicits a complex triphasic effect on the RMP of gallbladder neurons with the predominant effect being a long-lasting hyperpolarization. In addition, PGE2 attenuates synaptic input to the gallbladder neurons, an important site of neuromodulation in this organ. Our results suggest that PGE2 may be involved in a reduction in contractility at the level of the intramural ganglia and over the long term may contribute to the gallbladder stasis seen in pathophysiological conditions.

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