Modulation of Nicotinic AChR Channels by Prostaglandin E2 in Chick Sympathetic Ganglion Neurons

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Tan, Wen, Chuang Du, Steven A. Siegelbaum, and Lorna W. Role. Modulation of nicotinic AChR channels by prostaglandin E2 in chick sympathetic ganglion neurons. J. Neurophysiol. 79: 870–879, 1998. The effects of prostaglandin E2 (PGE2) on the activity of nicotinic AChR channels in cultured chick sympathetic ganglion neurons. In whole cell recordings, PGE2 (25 nM) inhibited significantly the ACh-evoked macroscopic current. In cell-attached patch recordings, PGE2 significantly inhibited single AChR channel currents as a result of a decrease in the frequency of channel opening, with no change in open time and conductance. PGE2 did not alter the extent or rate of agonist-induced desensitization of the AChR channels. These effects are specific since the related compound PGD2 had no effect on AChR channel function. Because there is an abundant endogenous production of PGE2 within sympathetic ganglia in response to certain stimuli, the inhibition of AChR channel function by PGE2 could serve an important role to modulate synaptic transmission in the sympathetic nervous system.

INTRODUCTION

Prostaglandin E2 (PGE2) is one of the major biologically active metabolites of arachidonic acid in the body. Although it is well established that PGE2 plays an important role in regulating autonomic nervous system function, the mechanisms by which PGE2 alters sympathetic output is less clear (see Gullner 1983; Hedqvist 1977; Shimizu and Wolfe 1990 for reviews). Previous efforts to explore the action of PGE2 on sympathetic function focused on changes in catecholamine release from primary sympathetic projection neurons and chromaffin cells. These studies produced conflicting reports, however, with PGE2 either inhibiting or enhancing catecholamine release depending on the preparation studied and the approaches used (see Shimizu and Wolfe 1990).

Recent biochemical studies revealed that PGE2 is the most abundant endogenous prostaglandin released from rat sympathetic ganglia (Gonzales et al. 1991). Thus another locus of regulation might be at the autonomic ganglion itself. Studies of the direct effects of PGE2 on cholinergic transmission within sympathetic ganglia have received little prior attention, however. In the present report, we demonstrate that embryonic chick sympathetic neurons also synthesize and release PGE2 in response to specific stimuli and present evidence that PGE2 decreases nicotinic receptor activity in the primary sympathetic neurons. Our results show that nanomolar concentrations of PGE2 are sufficient to inhibit significantly the fast inward nicotinic currents activated by applied acetylcholine (ACh). Inhibition of ACh-evoked currents by PGE2 is mediated by a selective decrease in the frequency of nicotinic AChR channel opening with no change in channel open duration or single-channel conductance. Our demonstration of stimulated-release of PGE2 from chick sympathetic ganglia neurons in conjunction with its effects on ganglionic transmitter function suggests a novel mechanism for the control of sympathetic outflow.

METHODS

Cell preparation

Sympathetic ganglion neurons were prepared and maintained in vitro as previously described (Role 1984). Briefly, the lumbothoracic or cervical region of the sympathetic chain was dissected from embryonic chickens (embryonic day 11–13), dispersed to individual cells, and plated on polyornithine-coated dishes at a density of 2.5–5 × 10^5 cells/35-mm dish. The cultures were maintained at 37°C in Dulbecco’s modified Eagle’s medium (GIBCO) supplemented with 5% chicken embryo extract, horse serum, penicillin, glutamine, and nerve growth factor (0.01 μg/ml) for 4 to 7 days before recording. For biochemical experiments, neurons were plated at a density of 1.3–1.7 × 10^6 cells/60-mm culture dish. In these experiments the suspension was typically γ-irradiated (≈5,000 rad) before plating to eliminate the proliferation of nonneuronal cells. Under these conditions, we cannot detect the presence of nonneural cells. Also we do not detect muscarinic responses from the cultured cells under the conditions of our experiments. Therefore all responses to ACh measured here reflect solely nicotinic ACh receptor-activated currents.

Current recording and data analysis

ACh-evoked currents were recorded at room temperature in either the whole cell or the cell-attached configuration by using a List EPC-7 amplifier. Patch pipettes were prepared from borosilicate glass and had resistances of 2–5 MΩ. In macroscopic current recording experiments, neurons were voltage clamped at –60 mV. Current responses were evoked by direct application of ACh (20 μM), either for 500 ms to determine the peak amplitude of the ACh-gated current or for 10–15 s to study the time course of AChR desensitization. The recording chamber was continuously perfused at 1–1.5 ml/min by a microperfusion system. The inflow of the microperfusion was placed 3–4 mm away from the recorded cell to allow rapid exchange of the local perfusate. Agonist was applied via a second pipette placed within 50 μm of the cell soma. This configuration optimized the rate of both delivery and washout of applied drugs.

MACROSCOPIC CURRENT ANALYSIS. The effects of drugs on peak currents evoked by nicotinic agonists were analyzed as follows. Three 500-ms long applications of agonist were applied to a cell, each separated by a 7–10 min interval. Although the initial peak of ACh-evoked current varied from cell to cell, the percent...
decline of the peak of subsequent responses was quite consistent. Prostaglandins (PGs) were applied after the first ACh response. To allow comparison between cells, the absolute amplitude of the second and third responses were normalized by the first response.

The half-time of AChR desensitization was determined from the time course of current decline in the continued presence of agonist. Previous studies have shown that a reliable estimate of the rate of AChR desensitization is provided by the ratio \((I_{\text{peak}} - I_t)/I_{\text{peak}}\) or \(I_t/I_{\text{peak}}\) (where \(I_{\text{peak}}\) is the peak current and \(I_t\) the current measured after 5 s of ACh application, see Valenta et al. 1993).

**RESULTS**

**Prostaglandin synthesis**

Neurons were incubated in two solutions and maintained at 37°C. Solution A contained (in mM) 150 NaCl, 1 MgCl\(_2\), 1 KCl, 2.5 CaCl\(_2\), and 10 HEPES, pH 7.2; solution B consisted of solution A plus 0.05% fatty acid free BSA (Sigma). Neurons previously maintained in vitro for 5–7 days (~1.5 × 10^5 neurons per 60-mm dish) were incubated with solution B for 1–2 min (to remove possible residual PGs) and then washed twice with solution A. Individual plates were then incubated for a period of 10 min with solution A alone, solution A containing NE (0.1 or 1 mM) and ascorbic acid (100 mM) or solution A containing other reagents (see results). Each condition was tested in duplicate or triplicate. In some experiments, the neurons were pretreated with indomethacin (10 μM; Sigma) for 20 min before incubation to block cyclooxygenase activity and hence the production of PGE\(_2\). At the end of the incubation, two volumes of ethanol were added to each plate and the neurons and media collected for extraction.

After ethanol extraction (~20°C, 4–12 h), the samples were centrifuged (10,000 × g for 15 min), the supernatants were collected and the ethanol in the samples evaporated under a stream of N\(_2\). The remaining solutions were acidified to pH 4 with HCI and the prostaglandins extracted by three sequential treatments with 2 × (v:v) ethyl acetate. After evaporation to dryness, the residue was resuspended in ELISA buffer (Cayman) for enzyme immunonassay. PGE\(_2\) concentrations were determined by ELISA as described by Farman et al. (1986) by using an enzyme immunoassay protocol and reagents from Cayman.

**PGE\(_2\) attenuates the peak ACh-evoked macroscopic current**

Figure 1 compares the macroscopic currents evoked by ACh (20 μM) under control conditions (Fig. 1A, top) and after PGE\(_2\) treatment (25 nM; Fig. 1B, bottom). In the absence of PGE\(_2\), stable ACh responses were evoked by repeated applications of agonist, with only a slight decline in peak current amplitude in response to the second and third ACh applications. However application of PGE\(_2\) for 3.5 min after the initial control response significantly decreased the amplitude of the peak ACh-evoked current (Fig. 1B). This decrease in the peak current is largely reversed after washout of PGE\(_2\) (Fig. 1B, trace 3).

These effects of PGE\(_2\) were consistently observed in a total of 17 experiments (Fig. 1C). Under control conditions the second and third responses to ACh were 91 ± 3.3% and 73 ± 8% of the first response, respectively. In contrast, during treatment with 25 nM PGE\(_2\), the amplitude of the ACh-evoked current was only 33 ± 3.8% of the initial response (P < 0.05). After washout of PGE\(_2\) for 7 min, the ACh response recovered on average to 60.1 ± 6% of the initial response (P < 0.05 compared with PGE\(_2\), no significant difference compared with control). Part of the incomplete recovery is probably the result of a prolonged component of desensitization (Valenta et al. 1993), evident in the decline of the ACh responses under control conditions. A similar inhibitory effect of PGE\(_2\) was observed in experiments with perforated patch recording. After treatment with PGE\(_2\), the amplitude of the ACh-evoked current was only 36 ± 4.5% of the initial response (P < 0.05, n = 4).

PGE\(_2\) had no effect on the resting potential, the holding current under voltage clamp at ~60 mV, or input resistance.
rate of AChR desensitization. This would be similar to the action of several neuropeptides on both central and peripheral, neuronal AChRs (Belcher and Ryall 1977; Boyd and Leeman 1987; Clapham and Neher 1984; Valenta et al. 1993). The possible effects of PGE2 on AChR desensitization were examined with a prolonged pulse of ACh (10 s), during which the macroscopic current declines markedly because of desensitization.

Figure 2 illustrates typical responses to repeated 10 s applications of ACh under control conditions (Fig. 2A) and after PGE2 treatment (Fig. 2B). Under control conditions, the second response to ACh is typically 30–40% less than the initial response (10 min interval between application), reflecting a maintained component of desensitization of the AChRs (see Boyd and Leeman 1987; Valenta et al. 1993). However the time course of decay of currents during the first and second responses is identical, as indicated by superimposing a scaled version of the second response on top of the first response (superimposed traces in Fig. 2A, bottom).

One possible mechanism for the decrease in the amplitude of ACh-evoked currents by PGE2 is an enhancement of the (i.e., no change in current in response to ± 20 mV voltage steps). Thus the action of PGE2 is relatively selective toward inhibition of the current evoked by ACh (see Fig. 4). The effect of PGE2 on ACh-evoked currents was examined further by altering the PGE2 concentration between 1 and 250 nM (n = 9). The ACh responses were 17% (n = 5), 45% (n = 4), or 43% (n = 5) of the initial response after treatment, with 1, 12.5, or 250 nM PGE2 respectively, indicating that the IC50 for PGE2 was below 1 nM.

PGE2 decreases the peak ACh-evoked current without altering the early phase of AChR desensitization

One possible mechanism for the decrease in the amplitude of ACh-evoked currents by PGE2 is an enhancement of the

![Figure 1](http://jn.physiology.org/)

![Figure 2](http://jn.physiology.org/)
Similarly, the rate of AChR desensitization during continuous agonist application was not altered by the presence of PGE$_2$, although the PGE$_2$ treatment did decrease the subsequent ACh-evoked response by 60% (Fig. 2B). The lack of effect of PGE$_2$ on the time course of desensitization during the ACh pulse is illustrated by the fact that currents evoked by ACh in the presence of PGE$_2$ are superimposable on the initial control response after amplitude scaling (Fig. 2B, bottom).

The results using 10 s long ACh pulses are summarized for 18 control and 19 PGE$_2$ treated neurons in Fig. 2C. On average, the peak response to a second ACh application was 68.7 ± 4.3% of the first response under control conditions versus 36.4 ± 4.5% of the first response in PGE$_2$ treated neurons ($P < 0.01$). In contrast, AChR desensitization during the pulse of ACh, estimated by the percent decrease of the current from its initial peak value ($I_{peak}$) after 5 s of continuous application of ACh ($I/I_{peak}$, see METHODS) was unchanged by PGE$_2$ (72 ± 4.3%; control and 65 ± 6.5% + PGE$_2$; $P > 0.05$). Furthermore, to minimize variations in desensitization rates among different cells, the fraction of current remaining at 5 s during the second ACh application was normalized by the fraction during the first ACh application. These normalized measures of desensitization were also not altered by PGE$_2$ (0.97 ± 0.03 in control vs. 0.94 ± 0.06 in presence of PGE$_2$, $P > 0.05$; Fig. 2C).

**Effects of PGE$_2$ on ACh-evoked single-channel currents**

To investigate further the mechanism by which AChR currents are inhibited by PGE$_2$, its action was studied on single AChR channel currents in cell-attached patches. In these experiments, channels were activated by including 2 μM ACh in the patch pipette, a concentration that results in little desensitization of either central or peripheral AChR channels (Brussard et al. 1994; Downing and Role 1987; Listerud et al. 1991) and PGE$_2$ was applied to the bath solution. As illustrated in Fig. 3, PGE$_2$ consistently inhibited the AChR channel current in the cell-attached patches. Three sets of nonconsecutive AChR channel currents from an individual patch recording are shown in Fig. 3A, recorded before application of PGE$_2$ (control), in the presence of 25 nM PGE$_2$, and 1–3 min after removal of PGE$_2$ (Wash). The overall level of AChR channel-opening frequency is greatly reduced after application of PGE$_2$ to the extrapatch membrane and largely recovers within a few minutes after washout of drug.

To quantify the effect of PGE$_2$ on single AChR channel currents, the mean current ($I$) during 10 s time windows is plotted versus time during the experiment (Fig. 3B). Treatment with PGE$_2$ decreased the mean current from about $8 \times 10^{-5}$ pA to about $2 \times 10^{-5}$ pA. The mean current then largely recovered on washout of PGE$_2$. Both the inhibition and the recovery of AChR channel activity occur fairly slowly, requiring one or more minutes. Figure 3C summarizes the results from six experiments in which mean AChR channel current was measured continuously under control conditions and in the presence of PGE$_2$. Because of the slow and variable onset and recovery of the effects of PGE$_2$, a 2 min window of channel activity was averaged beginning one minute after exposure to PGE$_2$ (+PGE$_2$) and 3–6 min after the washout of PGE$_2$ (recovery). On average PGE$_2$ treatment reduced the mean current by 64.5 ± 5% from the control level of channel activity ($n = 6$, $P < 0.05$). The mean currents recovered to 76 ± 8% of control after PGE$_2$ washout ($n = 5$).

Thus the effects of bath applied PGE$_2$ on AChR channel activity in cell-attached patches quantitatively matches the inhibitory effects of bath applied PGE$_2$ on macroscopic currents (Fig. 1B). Because the channels in the cell-attached patches were not directly exposed to PGE$_2$, it is unlikely that PGE$_2$ directly interacts with the AChRs. More likely, the inhibition of nicotinic (nAChR) activity by PGE$_2$ is mediated by a specific membrane receptor for the prostaglandins coupled to an intracellular second messenger.

**PGE$_2$ alters the probability of opening but not the single-channel current amplitude or burst duration**

To determine at which level single AChR channel function is altered by PGE$_2$, we analyzed the single-channel records for changes in single-channel current amplitude, frequency of channel opening and burst duration.

**Analysis of nAChR-channel current amplitudes.** ACh activates four nAChR channel subtypes distinguished by their conductance, open time kinetics, pharmacology, and developmental stage of expression in chick sympathetic neurons (Moss et al. 1989; Moss and Role 1993). Probably because of our use of relatively early stage embryos in these experiments (E11–E13), we routinely observed only two
of these amplitude classes of channels (as can be seen in Fig. 3A), with conductances of ~45 and 25 pS, respectively (assuming an average cellular resting potential of ~50 mV). The two channels were distinguished with the use of an "all points" current amplitude histogram (see Jaramillo and Schuetze 1988 and METHODS).

Analysis of records such as the one illustrated in Fig. 3 before and after treatment with PGE2 indicates that PGE2 has no detectable effect on the single-channel current amplitude of either of these nAChR channels (Fig. 4, A and B). The control amplitude histograms are well fit by the sum of two Gaussian functions with peaks at ~2.6 ± 0.3 pA and ~4.96 ± 0.37 pA, respectively (Fig. 4A, mean ± SD). Analysis of the current amplitudes of the nAChR channels from the same patch after treatment with PGE2 reveals a similarly good fit by two similar Gaussian functions, with peak amplitude at ~2.57 ± 0.26 pA and ~4.85 ± 0.32 pA (Fig. 4B, mean ± SD). Thus although there were many fewer openings after application of PGE2, the amplitudes of the open-channel currents were unchanged.

These data are summarized from six such analyses of current amplitude histograms in Fig. 4C. On average, the amplitude of the larger conductance class of nAChR channels was ~4.79 ± 0.21 pA and ~4.83 ± 0.25 pA, before and after PGE2, respectively (n = 6, P > 0.05). The average amplitude of the smaller conductance class was ~2.86 ± 0.17 pA (control) and ~2.7 ± 0.14 pA (+PGE2; n = 6, P > 0.05).

**ANALYSIS OF nAChR-CHANNEL OPEN BURST DURATION.** Possible effects of PGE2 on the open burst duration of either of the two amplitude classes of channels were analyzed next. Each conductance class was studied separately using the criterion that a burst consisted of a group of openings separated by closed times no longer than 3 ms. Burst distribution histograms required two exponential functions for adequate fitting. PGE2 altered neither fast nor slow burst duration time constants for either conductance class of channels. One example of the lack of effect of PGE2 on open burst distributions for the larger conductance class of nAChR channel is illustrated in Fig. 4, D and E. In control conditions, the open burst distribution was fit by two exponential functions with fast (τf) and slow (τs) time constants of, respectively, 1.06 ms (relative amplitude of 0.79) and 3.7 ms. In the presence of PGE2 the fast and slow time constants were essentially unchanged, with values of 1.04 ms (0.89 relative amplitude) and 3.87 ms, respectively.

A summary of such burst distribution analyses for the large conductance class of nAChR channels is presented in Fig. 4F for five such experiments. On average, τf was equal to 0.87 ± 0.15 ms under control conditions versus 0.73 ± 0.16 ms after treatment with PGE2 (P > 0.05). τs was equal to 3.05 ± 0.5 ms under control conditions versus 2.94 ± 0.53 ms after treatment with PGE2 (P > 0.05).

Analyses of the open burst kinetics of the smaller amplitude nAChR channel was confined to the one experiment in which there was a sufficient number of reliable measurements both before and after PGE2 treatment. In this experiment, PGE2 had no effect on the burst kinetics (control: τf = 0.69 ms, τs = 2.94 ms; +PGE2: τf = 0.51 ms, τs = 2.82 ms). The mean burst duration of the smaller class of nAChR channels was also estimated from the arithmetic mean of all open bursts and revealed a control value of 2.27 ± 0.46 ms versus 2.25 ± 0.35 ms after PGE2 treatment (P > 0.05; n = 6).

**ANALYSIS OF FREQUENCY OF nAChR OPEN BURSTS.** As PGE2 had no apparent effect on either single-channel current amplitude or open burst duration, it was likely that PGE2 acted to decrease the frequency of AChR channel open bursts. Channel open burst frequency was determined by averaging the number of open bursts of each nAChR channel amplitude class during three minutes of recording in the absence or presence of PGE2. In the absence of PGE2, the average open burst frequency of the larger and smaller conductance class of nAChR channels was 41.5 ± 24/min (n = 6)...

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**FIG. 4.** PGE2 decreases channel opening frequency without altering single-channel amplitude or open burst time. A-C: analysis of single AChR channel amplitude. All points current amplitude histograms for channel openings from a cell-attached patch before (A) and after (B) bath application of PGE2. Superimposed on each histogram is sum of 2 Gaussian curves. V_r = resting potential = 50 mV. Data sampled at 50 µs intervals, 20 bins/pA. C: summary of single-channel amplitude determinations in cell-attached patches in absence (solid bars) and presence (hatched bars) of PGE2. D-F: analysis of AChR channel open burst duration. D and E: representative burst duration distributions of larger amplitude class of channels in a patch gated by AChR (D) and after (E) bath application of PGE2 (25 nM). Insert: initial part of distribution on a faster time scale. Distribution of channel openings under control conditions and during PGE2 are fit by 2 exponential curves. F: summary of open burst distribution analysis of 45 pS large channel before (control, solid bars) and after (hatched bars) PGE2 treatment (n = 5).

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to the initial response. The normalized current responses were $0.91 \pm 0.04$ for control, $0.89 \pm 0.08$ for neurons treated with PGD$_2$, and $0.37 \pm 0.04$ for neurons treated with PGE$_2$. Thus PGD$_2$ had no significant effect on the ACh-evoked current ($P > 0.05$), whereas PGE$_2$ treatment diminished the peak current significantly (compared with the neurons treated with control media or PGD$_2$; $P < 0.05$). Similar results were obtained using a 10-fold higher concentration of the prostaglandins. These data indicate that the inhibitory effects of prostaglandins on nAChRs were specific for PGE$_2$ relative to PGD$_2$.

Release of PGE$_2$ from cultured chick sympathetic neurons

As an autacoid, PGE$_2$ is mostly likely to alter cellular function near the site of its synthesis and release. To test whether or not PGE$_2$ might be synthesized by sympathetic ganglion neurons themselves in response to local stimuli, sympathetic neurons maintained in vitro were incubated under control conditions or in the presence of hormones or transmitters. The role of cyclooxygenase in both basal and stimulated PGE$_2$ release was tested by pretreating neurons with indomethacin (a cyclooxygenase blocker). After 5–10 min of incubation of sympathetic neurons under control conditions, PGE$_2$ synthesis ranged from 82 ± 112 pg/1.2 $\times$ $10^3$ + 0.3 $\times$ $10^5$ neurons. In a series of experiments in which PGE$_2$ synthesis was measured in neurons treated with ACh (0.01–0.2 mM), dopamine (100 $\mu$M), histamine (10–100 $\mu$M), bradykinin (10–100 $\mu$M), vasopresin (100 $\mu$M), muscarine (10–100 $\mu$M), nicotine (0.01–0.1 $\mu$M), and norepinephrine, only the later produced a reproducible and sig-

PGD$_2$ does not alter the ACh-evoked current in sympathetic neurons

To examine whether modulation of the AChR is selective for PGE$_2$, we examined the action of PGD$_2$, a prostaglandin that is similar in both biological activity and structure to PGE$_2$ (Shimizu and Wolfe 1990), on channel function. To compare the inhibitory effects of PGE$_2$ with PGD$_2$, we examined ACh-evoked macroscopic currents in neurons from three sets of sibling cultures, using a procedure identical to that used above to study the effects of PGE$_2$. In each neuron we evoked two sequential responses by brief applications of ACh (20 $\mu$M; 500 ms) with a 7-min interval between trials. During this interval the neurons were perfused either with control media or, for the final 3.5 min of this interval, with media containing PGD$_2$ (25 nM). The peak amplitudes of the subsequent ACh-evoked currents were then normalized

![Fig. 5. AChR channel opening frequency before and after PGE$_2$ application. Frequency of ACh-elicited channels was determined before (solid bar) and after PGE$_2$ treatment (hatched bars) for 3 min. Average number of openings per unit time (frequency) after PGE$_2$ treatment is plotted relative to channel opening frequency under control conditions ($n = 6$). Data after PGE$_2$ treatment plotted for all openings (all classes), 25-pS class alone, or 45-pS class alone.](http://jn.physiology.org/)

![Fig. 6. Synthesis and release of PGE$_2$ from sympathetic neurons. Dispersed sympathetic neurons incubated for 10 min under following conditions: Basal, control buffer with no additions; Indo, control buffer with 10 $\mu$M indomethacin (neurons were also pretreated with 10 $\mu$M indomethacin for 20 min); NE, control buffer plus 0.1 or 1.0 $\mu$M norepinephrine; Indo plus NE, indomethacin plus 0.1 mM NE. PGE$_2$ release was measured under each condition and normalized by basal release of PGE$_2$ from same set of cultures.](http://jn.physiology.org/)
significant increase in PGE\(_2\) production. These findings are summarized in Fig. 6.

One potential problem in measurements of prostaglandin levels is contamination by prostaglandins present in the culture media. To distinguish exogenous prostaglandins (PGs) from PGs produced by the sympathetic neurons, we compared PG levels in cultures incubated in the presence or absence of indomethacin, an inhibitor of cyclooxygenase that will block endogenous prostaglandin synthesis. Because the inclusion of indomethacin decreased basal release by \(~30\%\) (to 69 \pm 7\% of basal level; \(n = 3\)), this portion of PGE\(_2\) levels represents the endogenous synthesis by the sympathetic neurons.

NE stimulated synthesis of PGE\(_2\) in a dose-dependent manner, with levels increasing to 114 \pm 3\% (\(n = 3\)) and 130 \pm 5\% (\(n = 5\)) of basal levels with 0.1 and 1 mM NE, respectively (\(P < 0.05\)). This increase was largely blocked by indomethacin. PGE\(_2\) levels in indomethacin plus NE (0.1 mM) were 68 \pm 11\% of basal levels (\(n = 3\)). This is identical to the PGE\(_2\) levels measured in cultures treated with indomethacin in the absence of NE (\(P < 0.05\)). When PGE\(_2\) levels are expressed as the indomethacin-sensitive component, 0.1 mM NE caused an increase to 153\% of control and 1.0 mM NE caused an increase to 206\% of control. In summary, these data reveal a transmitter specific stimulation of PGE\(_2\) synthesis via a cyclooxygenase pathway in sympathetic neurons.

**DISCUSSION**

The results of the present study demonstrate that PGE\(_2\), an eicosanoid, inhibits the function of AChR channels in sympathetic ganglion neurons. The inhibition of the AChR channels seems to be specific and mediated through a second messenger. The first conclusion is supported by the finding that PGD\(_2\), a chemically similar compound to PGE\(_2\), did not show any inhibitory effects on the function of the AChR channels. The second conclusion is based on the cell-attached patch-clamp recordings in which PGE\(_2\), applied outside the patch electrode, attenuated the ACh-activated channel activity in the patch. Because we observe a similar extent of modulation in whole cell and perforated patch recordings, the unidentified second messenger system does not appear to rapidly wash out of the cell.

The inhibitory effects of PGE\(_2\) did not exhibit a dose-dependence within the concentration range tested (from 1 to 250 nM). At concentrations of 10 \(\mu\)M, we found that PGE\(_2\) enhances the AChR channel current (unpublished data). Dual effects of PGE\(_2\) were observed in other systems (Negishi et al. 1989; Shimizu et al. 1993). This may be the result of the coexistence of PGE\(_2\) receptor subtypes. Three PGE\(_2\) receptor subtypes were identified (reviewed by Coleman et al. 1987), which differ in terms of their affinity for PGE\(_2\), signal transduction pathways and physiological functions. To determine which receptor subtypes are involved in the present actions will require the availability of subtype specific agonists and an understanding of the particular second messengers related to these effects.

Previous studies have demonstrated that neuropeptides, such as substance P (SP) are also potent modulators of neuronal nicotinic AChR channels (Simmons et al. 1990; Valenta et al. 1993). In comparison, PGE\(_2\) may be more readily diffusible among cells within the ganglion because of its aliphatic side chains. Unlike SP, which can depolarize the membrane independent of its effects on AChRs (Dun and Minota 1981). PGE\(_2\), at the concentrations used in the present study, did not induce any changes in membrane potential while it modulated the ACh-evoked response.

There are several other differences in the modulatory effects on AChR channels between SP and PGE\(_2\). First, the effects of PGE\(_2\) are much slower than those of SP. Second, at the whole cell current level, SP inhibits ACh-evoked current by enhancing the rate of decay (or the rate of the early stage of desensitization) and slowing the late phase of recovery from desensitization. SP has little effect on the ACh-evoked peak current response (Role 1984). In contrast, PGE\(_2\) greatly attenuates the ACh-evoked peak current response, but has almost no effect on the rate of the early stage of desensitization. At the single-channel level, SP decreases the open time and frequency of opening of channels activated by a desensitizing concentration of ACh (7.5 \(\mu\)M; Simmons et al. 1990) but PGE\(_2\) affects only the opening frequency. Finally, the effects of SP were a function of agonist concentration. SP showed greater effects with high or desensitizing concentrations of ACh but had little effect when the AChR channels were activated by a low or nondesensitizing concentration of ACh (Valenta et al. 1993). In contrast, the effects of PGE\(_2\) did not depend on agonist concentration. PGE\(_2\) affected the activity of AChR channels to a similar extent whether the AChR channels were exposed to 20 \(\mu\)M ACh (in whole cell experiments) or to 2.5 \(\mu\)M (in single-channel experiments), a relatively nondesensitizing concentration of ACh.

Although we cannot exclude the possibility that PGE\(_2\) alters a late slow phase of AChR channel desensitization, such effects make an insignificant contribution to the inhibition of the peak current in our recordings. Thus, in our control studies (Fig. 1A), we showed that 7 min after the initial ACh-response, the peak response during a second pulse of ACh was equal to 90\% of the initial ACh response. In some experiments, PGE\(_2\) was not applied until 7 min (or longer) after the initial ACh response. At this time, the channels have largely recovered from slow desensitization, yet PGE\(_2\) still induced a large attenuation of the peak currents similar to that shown in Fig. 1B (data not shown).

Even though both SP and PGE\(_2\) act to inhibit AChR channel currents, the differences in their rate of action and effects on AChR kinetics may have important implications for their physiological roles in modulating synaptic transmission. SP, which rapidly enhances the rate of desensitization and shortens channel open time, may serve to rapidly modulate synaptic transmission by limiting the duration of the postsynaptic response to a single presynaptic impulse, especially when there is an accumulation of a high level of ACh. PGE\(_2\) may slowly modulate synaptic transmission by rendering the postsynaptic AChR channels less responsive to presynaptic impulses. Thus SP should act to inhibit preferentially high-frequency trains of stimuli whereas PGE\(_2\), once released, will inhibit transmission in a frequency independent manner.

The finding that the sympathetic neurons release PGE\(_2\) in a cyclooxygenase-dependent manner, indicates endogenous
synthesis of PGE₂ by the cultured sympathetic neurons. The activity of cyclooxygenase in these neurons may be regulated by NE because it enhances production of PGE₂. Evidence for a physiological role of PGE₂ in neuronal systems was provided by many prior studies. PGE₂ binding sites were found in different areas of the CNS (Dray et al. 1989; Pralong et al. 1990; Yumoto et al. 1986). Moreover it was shown that PGE₂ is the major arachidonic acid metabolite in several different types of neuronal tissues, including cortex (Bishai and Coceni 1992), dorsal root ganglia (Vesin and Droz 1991), and sympathetic ganglia (Gonzales et al. 1991). In vivo, there is a detectable amount of PGE₂ in cerebrospinal fluid in both animals and humans (Yergey et al. 1989).

In the present experiments, the high basal production of PGE₂ by the cultured chicken sympathetic neurons may be partially attributed to the excessive release of arachidonic acid resulting from the mechanical stimulation of experimental procedures. Therefore it may be more proper to evaluate the effects of NE by comparing the NE stimulated production of PGE₂ with the production of PGE₂ in the presence of indomethacin. In this case, stimulation by NE could almost double the production of PGE₂.

It was also shown that reduction of the endogenous synthesis and release of PGE₂ is correlated with hypertension (reviewed by Quilley et al. 1990). Furthermore it is known that cyclooxygenase blockers that inhibit the synthesis of prostaglandins are prohypertensive. Experimental evidence indicates that this effect is the result of a reduction of PGE₂ levels that interact with the sympathetic nervous system (Quilley et al. 1990). Two possible sites of action were proposed to mediate the effects of PGE₂ on sympathetic function. First, application of exogenous PGE₂ was shown to directly inhibit the release of catecholamine from the postganglionic nerve endings (Jackson et al. 1989). Second, PGE₂ could also activate autonomic afferent fibers and modulate the sympathetic outflow via the nucleus tractis solitarii-mediated autonomic reflex (Panzenbeck et al. 1989).

Most research has been focused on whether or not PGE₂ inhibits the release of NE from the sympathetic nerve terminals via a Ca²⁺-dependent mechanism. In sympathetic neurons PGE₂ inhibits Ca²⁺ influx and attenuates the voltage-gated Ca²⁺ currents (Ikeda 1992), thus reducing Ca²⁺-dependent NE release (Stjarne 1973). On the other hand, it was shown that PGE₂ enhances release of NE, intracellular Ca²⁺ levels and Ca²⁺ influx in adrenal chromaffin cells (Mochizuki-Oda et al. 1991; Yokohama et al. 1988). In cultured sympathetic neurons, we have found that PGE₂ at nanomolar concentrations can induce an increase in intracellular Ca²⁺ (data not show). Therefore it is not clear whether or not the hypotensive effect of PGE₂ can be explained by a direct inhibition of Ca²⁺-mediated NE release.

In the present study, we provide the first evidence that PGE₂ can act upstream of the release process, by modulating synaptic transmission in the ganglion through inhibition of the AChR channels. Because the ratio of synapse formation between preganglionic neurons and postganglionic neurons is estimated at 1 to 10 and, in some cases, 1 to 200 (Purves and Wigston 1983), modulation of synaptic outflow at the level of ganglionic transmission might be more efficient than modulation at individual sympathetic nerve endings.

What are the likely sources for PGE₂ if it does indeed play a significant role as a modulator of sympathetic function? One possibility is that is released from the sympathetic neurons themselves, perhaps in response to local NE release in the ganglion, serving as a negative feedback regulator of sympathetic transmission. It was reported that NE stimulates the release of PGE₂ from the neurons of the media eminence region (Ojeda et al. 1982). Among the drugs we tested for stimulation of PGE₂ production in sympathetic neurons, NE was the most effective. Although the main site of storage and release of NE is at the terminals of sympathetic ganglion neurons, several lines of evidence indicate that it may also be stored and released within the ganglion. First, NE was found to be stored in principal ganglion cell bodies and SIF (small intensively fluorescent) cells, which are believed to have modulatory functions on synaptic transmission (Libet 1980). Second, fluorescence histochemical studies show that there is an intraganglion network of NE containing varicose fibers (Dail and Barton 1983). This NE can be released on stimulation and this release is blocked by the NE-depleting effect of reserpine (Christ and Zitaglio 1984). Finally, isolated cervical ganglia, stimulation of the preganglionic nerve induces release of NE from the ganglia (Martinez and Adler-Graschinsky 1979). A significant amount of NE may also reach the ganglion cells via the circulation resulting from high concentrations of NE released from activated adrenal glands. The thick sheath of adventitia surrounding sympathetic ganglia imposes a diffusion barrier that could result in a much higher relative concentration of substances or ions within the ganglion (Dail and Barton 1983). It is possible that there is sufficient local NE to stimulate the production of PGE₂ within the ganglion. Finally, exogenous PGE₂ could also become accessible to sympathetic neurons via the rich blood supply and the significant glomerular structure within the ganglion (Dail and Barton 1983). As a relatively stable prostaglandin autocoid with the potent effects on AChR channels that we have demonstrated, PGE₂ is likely to be an important modulator of sympathetic nervous system function.

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