The Action of Prostaglandins on Ion Channels

Hans Meves*

Physiologisches Institut, Universität des Saarlandes, D-66421 Homburg-Saar, Germany

Abstract: Prostaglandins, in particular PGE₂ and prostacyclin PGI₂, have diverse biological effects. Most importantly, they are involved in inflammation and pain. Prostaglandins in nano- and micromolar concentrations sensitize nerve cells, i.e. make them more sensitive to electrical or chemical stimuli. Sensitization arises from the effect of prostaglandins on ion channels and occurs both at the peripheral terminal of nociceptors at the site of tissue injury (peripheral sensitization) and at the synapses in the spinal cord (central sensitization). The first step is the binding of prostaglandins to receptors in the cell membrane, mainly EP and IP receptors. The receptors couple *via* G proteins to enzymes such as adenylate cyclase and phospholipase C (PLC). Activation of adenylate cyclase leads to increase of cAMP and subsequent activation of protein kinase A (PKA) or PKA-independent effects of cAMP, e.g. mediated by Epac (=exchange protein activated by cAMP). Activation of PLC causes increase of inositol phosphates and increase of cytosolic calcium. This article summarizes the effects of PGE₂, PGE₁, PGI₂ and its stable analogues on non-selective cation channels and sodium, potassium, calcium and chloride channels. It describes the mechanism responsible for the facilitatory or inhibitory prostaglandin effects on ion channels. Understanding these mechanisms is essential for the development of useful new analgesics.

Key Words: Prostaglandin, cAMP, Epac, protein kinase A and C, dorsal root ganglion cells, neuroblastoma cells, ion channels, sensitization.

INTRODUCTION

Prostaglandins are potent and ubiquitous lipid messenger molecules. Arachidonic acid, released from phospholipids by the action of phospholipase A₂, is converted by the cyclooxygenase enzymes to prostaglandin H₂. Prostaglandin H₂ is, in turn, the precursor of multiple prostaglandins.

Prostaglandins have a variety of functions. The present review concentrates on the effects of prostaglandins on ion channels. Such effects have been mainly studied on nerve cells and, more recently, in heterologous expression systems.

An effect on ion channels underlies the well known sensitizing action of prostaglandins PGE₂ and PGI₂. They lower the firing threshold of sensory neurons and increase the number of action potentials elicited by a suprathreshold stimulus. Thereby, they “induce a state of hypersensitivity in which normally non-painful stimuli become painful – for example, the rubbing of clothes against sunburnt skin” (quoted from [94]). In recordings from isolated sensory neurons grown in culture, treatment with PGE₂ enhances the number of action potentials generated by exposure either to elevated levels of potassium or focally applied bradykinin (Fig. 1). Prostaglandins often lower the resting potential, but sensitization is not simply the consequence of the depolarization. It is also seen when the resting potential changes little [80, 53] or is brought back to its normal value by a hyperpolarizing current (Fig. 2), suggesting a direct effect of prostaglandins on Na⁺ or K⁺ channels.

The enzyme cyclooxygenase (COX) exists in two isoforms: COX-1 and COX-2 (for review, see [41]). COX-1 is constitutively expressed in most tissues throughout the

body. COX-2 has a restrictive pattern of distribution under normal conditions, yet is highly induced at sites of inflammation and cell proliferation. Substances which inhibit the cyclooxygenase enzymes, in particular selective COX-2 inhibitors, play an important role in the treatment of inflammation and arthritis. The effect of NS-398, a selective COX-2 inhibitor, on hippocampal slices is opposite to that of PGE₂: It reduces the frequency of action potentials elicited by a depolarizing current [19].

Prostaglandins bind to receptors on the cell surface which couple to G proteins. Various types of prostanoid receptors have been described: DP, EP, FP, IP and TP receptors (for review, see [15, 24, 77, 113]). PGE₂ binds to EP receptors and only at high concentrations to IP receptors. PGE₁, PGI₂ and most of its stable analogues bind to IP and EP receptors. Both EP and IP receptors are important in inflammatory pain [10]. Agonists and antagonists for the various prostanoid receptors have been described, but truly selective ligands with high potency are rare. The affinity of prostanoids and selective agonists for the mouse and rat prostanoid receptors has been studied in detail [12, 58]. Agonists acting on human prostanoid receptors have also been investigated [1, 120]. In addition to the classical agonists, a new generation of specific EP agonists and antagonists has been developed by Ono Pharmaceutical Co. Ltd.. Chemical formulae, EC₅₀ values and K_i values are found in [123] and [113]. For IP receptors, the stable PGI₂ analogue cicaprost has long been regarded as the “standard IP receptor agonist” [121] because of its high selectivity for IP receptors. A novel IP agonist is the compound ONO-54918-07 [47, 76].

A) PGE₂ ACTING ON ION CHANNELS VIA EP RECEPTORS

Non-Selective Cation Channels

PGE₂ can depolarize cells by activating non-selective cation channels. In voltage-clamp experiments, it elicits an

*Address correspondence to this author at Physiologisches Institut, Universität des Saarlandes, D-66421 Homburg-Saar, Germany; Tel: +49 6841 1626479; Fax: +49 6841 1626468; E-mail phhmev@uniklinik-saarland.de

Fig. (1). Examples for the sensitizing action of PGE₂. Treatment with PGE₂ (1 μ M in A, 0.1 μ M in B) enhances the number of action potentials elicited in rat sensory neurons by 100 nM bradykinin (Bk) (A) or 50 mM K⁺ (B). From [79] and [5].

inward current. Examples are shown in Fig. 3. At colon crypt cells the depolarization starts at 10 nM and increases only slightly at higher concentrations (Fig. 3A). On voltage-clamped nerve cells of the nucleus tractus solitarius, PGE₂ markedly affects the current-voltage curve at voltages more negative than -30 mV (Fig. 3B): It augments the inward current, shifts its reversal potential in the positive direction and increases the slope of the curve. The effect of PGE₂ on the membrane current of spinal cord neurons is illustrated in Fig. 3C and D and is mimicked by other EP receptor agonists. The depolarization and the inward current are not affected by changing the internal or external Cl⁻ concentration or by replacing the external Na⁺ with other monovalent cations like K⁺, Rb⁺ or Cs⁺, but vanish when all cations are replaced by N-methyl-D-glucamine.

It is tempting to speculate that the PGE₂-activated non-selective cation channels in the experiments of Fig. 3 belong to the superfamily of TRP (=transient receptor potential) channels (for review, see [23]). Such channels are present in most cells, e.g. in dorsal root ganglion (DRG) cells (see

Table 1 of [117]). TRPV1 channels in DRG cells are receptors for the vanilloid capsaicin. They are opened by capsaicin, resulting in an inward current carried by Na⁺ and Ca²⁺ and reversing at approximately 0 mV. Capsaicin sensitivity is completely absent in TRPV1 knock-out mice. The capsaicin-induced inward current is markedly enhanced by prostaglandins (Fig. 4). This phenomenon was first observed by Pitchford and Levine [85] and Lopshire and Nicol [64] and recently studied by Narumiya's group [76]. Fig. 4A shows the transient inward current following focal application of capsaicin. A second application after a 10 min exposure to PGE₂ produces a larger current. After 25 min exposure the sensitization by PGE₂ has disappeared. The inward current is accompanied by a rise in internal [Ca²⁺] (Fig. 4B). Normally, a second application 10 min later has little effect (Fig. 4B top). It becomes, however, highly effective after pretreatment with 0.25 μ M PGE₂ (Fig. 4B bottom). Likewise, augmentation of the Ca²⁺ transient by PGE₂ has been observed in vagal sensory neurons [36]. Potentiation of the capsaicin-activated current by PGE₂ is

Fig. (2). Sensitization of a colonic dorsal root ganglion cell by PGE₂. Bath application of 1 μ M PGE₂ decreases the action potential threshold (top) and increases the number of action potentials elicited by a suprathreshold stimulus (bottom). The resting potential was -57 mV before PGE₂. Hyperpolarizing current was used to maintain the membrane potential at -57 mV after PGE₂ application. From [34].

Fig. (3). Effect of PGE₂ on non-selective cation channels. A: Membrane potential of rat colon crypt cells at different PGE₂ concentrations. From [104]. B: Voltage-clamp recording from neuron of the rat nucleus tractus solitarius with ramp command voltage (-100 to +20 mV in 1.8 s) in control and in 0.1 μM PGE₂. From [68]. C and D: Effect of PGE₂ and other EP receptor agonists on the membrane current of voltage-clamped neurons in rat spinal cord slices (holding potential -70 mV, agonist concentration 10 μM in C). From [4].

Table 1. EP Receptor Subtypes Mediating the PGE₂ Effect on Different Preparations

Receptor	Preparation	Effect	Mimicked by	Reference
EP1/3	melanotrophs	inhibition of I _{Ca}	sulprostone	[111]
EP1/4	TRPV1 channel and dorsal root ganglion	enhancement of capsaicin effect	ONO-DI-004	[76]
EP2	spinal dorsal horn	depolarization	butaprost	[4]
EP2	spinal dorsal horn	block of inhibitory glycine receptors	butaprost	[3]
EP2	nodose and jugular ganglion	action potential firing	butaprost	[59]
EP2/4	nodose ganglion	increase in TTX-R I _{Na}	ONO-AE1-259 ONO-AE1-329	[67]
EP2/4	osteoclasts	activation of I _{Cl}	ONO-AE1-259 ONO-AE1-329	[83]
EP3	dorsal raphe nucleus	depolarization	M&B 28767	[74]
EP3	paratracheal ganglion	inhibition of I _{Ca}	sulprostone	[48]
EP3	trigeminal ganglion	inhibition of I _{Ca}	ONO-AE-248	[14]
EP3C/4	dorsal root ganglion	cAMP increase	*	[108]
EP4	supraoptic nucleus	depolarization	ONO-AE1-329	[100]

* blocked by antisense oligonucleotides.

Fig. (4). Sensitization of TRPV1 channels by prostaglandins. Records from mouse DRG cells, except record C which is from a HEK293 cell expressing rat TRPV1 and mouse EP1 receptor. A: PGE₂ enhances the amplitude of the inward current elicited by capsaicin. From [64]. B: PGE₂ augments the Ca²⁺ signal produced by 20 nM capsaicin (calibration 0.2 ΔF/F and 150 s). From [44]. C: PGE₂ enhances the inward current elicited by capsaicin in a HEK293 cell coexpressing TRPV1 with EP1. D: The EP1 agonist ONO-DI-004 mimics the PGE₂ effect, the EP1 antagonist ONO-8713 prevents it. E: PGI₂ and the IP agonist ONO-54918-07 also enhance the capsaicin effect. C-E from [76].

also seen in human embryonic kidney-derived HEK293 cells expressing rat TRPV1 and mouse EP1 receptor (Fig. 4C), but not with other mouse EP receptors. The specific EP1 agonist ONO-DI-004 mimics the PGE₂ effect and the EP1 antagonist ONO-8713 prevents it (Fig. 4D). PGI₂ and the specific IP agonist ONO-54918-07 act in a similar way to PGE₂ (Fig. 4E). PGE₂ (10 nM) enhances also heat-activated non-selective cation current in a subpopulation of cultured rat DRG cells [87]. Furthermore, PGE₂ (10 μM) sensitizes mechanosensitive cation channels of cultured DRG neurons by shifting the relation between channel activity (NP₀) and pressure to smaller pressures [20].

A special type of non-selective cation current is the hyperpolarization-activated current I_h. In a subpopulation of cultured guinea-pig neurons, 1 μM PGE₂ produces a small (18%) increase of its amplitude and a small shift (+4 mV) of its voltage dependence [46].

Sodium Channels

The action of PGE₂ on tetrodotoxin-resistant (TTX-R) Na⁺ currents in DRG cells offers a ready explanation for the sensitizing, hyperalgesic effect of PGE₂ and has therefore

received much attention (for review, see [61, 122]). In addition to TTX-sensitive Na⁺ channels and multiple types of Ca²⁺ channels, nociceptive DRG cells express two types of slowly gated TTX-R Na⁺ channels, Na_v1.8 (=PN3, SNS) and Na_v1.9 (=PN5, NaN) channels (for details, see [92]). The major transient TTX-R channel isotype is Na_v1.8 while persistent TTX-R Na⁺ currents which inactivate slowly and incompletely are probably flowing through Na_v1.9 channels. The distribution of the sensory neuron-specific Na_v1.8 channels is restricted mainly to those subpopulations of DRG cells that give rise to unmyelinated C-fibres. As illustrated in Fig. 5, 1 μM PGE₂ enhances the TTX-resistant Na⁺ current in DRG cells by shifting its activation curve to more negative potentials and increasing its slope. This will increase the excitability of sensory neurons. The effect is observed on cutaneous and colonic DRG cells and also on pulmonary DRG neurons [60]. There are, however, several striking differences between cutaneous and colonic neurons with respect to PGE₂-induced changes in resting potential and excitability [34]. The importance of Na_v1.8 channels is emphasized by the finding [57] that PGE₂-induced hyperalgesia is inhibited by antisense oligonucleotides directed against Na_v1.8 channels. The PGE₂ effect on

Fig. (5). PGE₂ (1 μ M) increases the TTX-resistant Na⁺ current I_{Na} of dorsal root ganglion (DRG) cells and shifts the Na⁺ conductance vs. voltage curve g(V) to the left. A: I_{Na}(V) curves of neonatal rat DRG cells in control (○) and 3 (●) or 5 min (■) after application of PGE₂. B: g(V) curves for the experiment in A. A and B from [26]. C: I_{Na}(V) curves of rat DRG cells before (■) and after (●) application of PGE₂. Inset shows currents at V=0 mV. D: g(V) curves before (■) and after (□) PGE₂ and steady-state inactivation curve (circles) from a similar experiment. C and D from [31]. E: Effect of PGE₂ on the peak I_{Na} of a colonic rat DRG cell recorded at V=0 mV. Inset shows currents at times indicated by 1 and 2. F: g(V) curves from the experiment in E. E and F from [33]. All measurements in the presence of TTX (50 nM – 1 μ M).

Na_v1.9 channels was studied in both Na_v1.8-null and wild-type mice [93]. In these experiments, treatment with 10 μ M PGE₂ for 1 h increased the persistent TTX-R Na⁺ current attributed to Na_v1.9; the increase will lead to depolarization and augmented firing of spikes. A 5 min exposure to PGE₂ has no effect on Na_v1.9 [6]. Other inflammatory mediators like adenosine and serotonin have been shown to modulate the activity of TTX-R Na⁺ current in a manner similar to that seen for PGE₂. TTX-R Na⁺ channels are therefore drug targets for pain therapy [25].

Potassium Channels

Possibly, the enhancement of excitability by PGE₂ results also in part from the suppression of the delayed rectifier current. In DRG cells of embryonic rats, 1 μ M PGE₂ produces a time-dependent suppression of I_K [80]. After a 20 min exposure I_K at +60 mV is reduced by 48%. PGE₂ (1 μ M) inhibits the I_K of neonatal rat DRG neurons by only 10% [26] and the I_K of primary cultured hippocampal neurons by 22.5% [19]. A 50% inhibition of I_K by 10 μ M PGE₂ has been reported for T-lymphocytes [7]. In vascular smooth muscle

cells, a 50% inhibition of I_K requires 3.5 μM PGE_2 [89]. All these observations can be summarized by saying that PGE_2 concentrations between 1 and 10 μM inhibit I_K by 50% or less. Much greater PGE_2 sensitivity has been reported for the lipopolysaccharide-induced K^+ outward current in cultured rat microglia: 50% inhibition by 10 nM, 80% inhibition by 100 nM PGE_2 [17].

PGE_2 (100 nM) almost totally blocks the Ca^{2+} -activated K^+ current which gives rise to a slow, Ca^{2+} -dependent spike after-hyperpolarization in rabbit nodose neurons [118]. By contrast, 5 nM PGE_2 activates large conductance Ca^{2+} -activated K^+ channels from otherwise silent patches in human osteoblast bone cells [75]. PGE_2 (10 μM) also activates G protein-gated inwardly rectifying K^+ (GIRK) channels heterologously expressed in rat superior cervical ganglion [91].

Calcium Channels

Depending on the preparation studied, 1 μM PGE_2 either enhances or inhibits the hva (=high-voltage-activated) Ca^{2+} current I_{Ca} . On cultured chicken DRG neurons held at $V = -60$ mV and pulsed to +10 mV, peak I_{Ca} is 1.8fold increased by 1 μM PGE_2 [78]. On other preparations a strong inhibition is observed. Fig. 6 illustrates some of these results. PGE_2 produces a marked reduction of the sympathetic neuron Ca^{2+} current accompanied by a pronounced slowing of its rising phase (Fig. 6A and B). In paratracheal ganglion cells, nanomolar concentrations of PGE_2 are sufficient for a clear inhibition of I_{Ca} ($\text{IC}_{50} = 6.4$ nM) and this effect is likewise

observed with various prostanoid agonists (Fig. 6C). The inhibitory effect of PGE_2 in concentrations ≥ 10 nM in mouse trigeminal neurons is also mimicked by an EP_3 agonist (Fig. 6D). (See below for a full discussion of Fig. 6C and D). The slowing of the Ca^{2+} current rising phase, conspicuous in Fig. 6A, was less pronounced or absent in the experiments of Fig. 6C and D. Only a weak (11%) inhibition of I_{Ca} by 1 μM PGE_2 has been seen on hippocampal CA1 pyramidal neurons [19]. Inhibition of I_{Ca} by PGE_2 is not only seen in nerve cells. In rabbit carotid body chemoreceptor cells [35] and rat melanotrophs [111] the inhibition of I_{Ca} observed with 0.3 or 1 μM PGE_2 is 54 and 35%, respectively.

Chloride Channels

In rat osteoclasts, PGE_2 (>10 nM) stimulates an outwardly rectifying Cl^- current in a concentration-dependent manner and causes a long-lasting depolarization [83]. Likewise, PGE_2 (10 μM) activates the Cl^- current in pigmented ciliary epithelial cells [29].

Synaptic Channels

In contrast to the activation of Cl^- currents just mentioned, PGE_2 partially inhibits the glycine-operated Cl^- influx and the resulting inhibitory postsynaptic currents at synapses in the rat spinal cord [3]. PGE_2 acts *via* the EP_2 receptor and PKA on the $\alpha 3$ glycine receptor [3, 40, 127]. No effect of PGE_2 on other inhibitory or excitatory spinal synapses, operated respectively by GABA, AMPA or NMDA, was seen [3]. Another study on rat spinal cord [4] also failed to observe an effect of PGE_2 on glutamatergic

Fig. (6). Inhibition of hva (=high-voltage-activated) Ca^{2+} currents by PGE_2 and various EP agonists. A: Leak-subtracted Ca^{2+} currents of a rat superior cervical ganglion cell in control and in 1 μM PGE_2 . B: Current-voltage curves from the experiment in A in control (○) and in PGE_2 (●, current 10 ms after start of pulse; ■, rapid component of Ca^{2+} current rising phase determined by double exponential fit). A and B from [45]. C: Concentration-response curves for the inhibition of hva Ca^{2+} current of rat paratracheal ganglion cells by various prostanoid agonists. Peak Ca^{2+} currents were measured with pulses to +10 or +20 mV and expressed as fraction of control current. From [48]. D: Inhibition of hva Ca^{2+} current of Type 1 (■) and Type 2 (▲) mouse trigeminal neurons by the EP₃ agonist ONO-AE-248. From [14].

excitatory postsynaptic currents (EPSCs) whereas in the mouse spinal cord a long-lasting facilitation of EPCPs by PGE₂ was found [72]. PGE₂ seems also able to modulate synaptic transmission in the sympathetic nervous system. In cultured chick sympathetic ganglion neurons, PGE₂ (25 nM) inhibits significantly the fast inward current flowing through acetylcholine-activated (AChR) channels [110]. The effect results from a decrease in the frequency of channel opening with no change in open time and conductance. PGE₂ (0.1–1 µM) also reduces the amplitudes of both fast and slow EPSPs in guinea-pig gallbladder ganglia [49].

Which Types of EP Receptor are Involved?

To answer this question selective agonists for the various EP receptors are required. Until recently, only substances with limited selectivity or low potency were available. Examples of such drugs are sulprostone which binds to both EP1 and EP3 receptors with high affinity and butaprost, the classical EP2 receptor agonist, which suffers from low potency. As mentioned above, recently a new generation of highly selective, potent agonists for the four EP receptors has been developed: ONO-DI-004 (EP1 agonist), ONO-AE1-259 (EP2 agonist), ONO-AE-248 (EP3 agonist) and ONO-AE1-329 (EP4 agonist) [123]. The four new receptor agonists – even at concentrations 1000 times higher than their respective K_i values – have little effect on Chinese hamster ovary (CHO) cells expressing other EP receptors.

Examples of experiments using different EP agonists are illustrated in Fig. 3C and D, Fig. 4D and Fig. 6C and D. As shown in Fig. 3C and D, the effect of PGE₂ on the membrane current of spinal cord neurons is mimicked by the EP2 agonist butaprost and the non-selective EP agonist 19(R)-hydroxy PGE₂, but not by the EP1/EP3 agonist sulprostone and the EP1 agonist 17-phenyl trinor PGE₂. By contrast, sulprostone, the TP ligand STA₂ and the highly selective EP3 agonist ONO-AE-248 potently inhibit the Ca²⁺ current of nerve cells as does PGE₂ (Fig. 6C and D). In Fig. 4D, the EP1 agonist ONO-DI-004 mimics the potentiating effect of a short (1.5 min) PGE₂ application on TRPV1 channels. Data obtained by such experiments on various preparations are collected in Table 1. A study with antisense oligonucleotides is also included. As can be seen, all four receptor subtypes EP1, EP2, EP3, its splice variant EP3C and EP4 are found.

As shown in Table 1, the potentiating effect of PGE₂ on the capsaicin-induced current through TRPV1 channels [76] is mediated by EP1 (see Fig. 4D) as well as EP4 receptors. The pathway downstream of EP1 involves PKC and PLC, the pathway *via* EP4 causes PKA activation and comes only into play during a fairly long (6.5 min) PGE₂ application (see below). Stimulation of EP1 can also cause a robust increase in [Ca²⁺]_i as demonstrated on Chinese hamster ovary (CHO) cells expressing the mouse EP₁ receptor [54] (for details, see below). EP2 receptors mediate the PGE₂ effects on membrane potential and glycine receptors in rat spinal dorsal horn [3, 4]. Both EP2 and EP4 are known to activate adenylate cyclase *via* G protein G_s. Thus, the experiments described in [67] support the view that the increase in TTX-R I_{Na} by PGE₂ is caused by an increase in cAMP level. The same is true for the stimulation of outwardly rectifying Cl⁻

current in osteoclasts observed in [83]. The situation is less clear with regard to EP3 which according to Table 1 seems to be the receptor subtype responsible for inhibition of I_{Ca}. EP3 receptors and their isoforms generally inhibit cAMP generation *via* G_i, but additional signaling mechanisms have also been described. They include stimulation of adenylate cyclase *via* G_s, Ca²⁺ mobilization *via* G_q and signaling through the small G protein Rho (for literature, see [15, 113]). Any of these mechanisms could be responsible for the EP3-mediated PGE₂ effects (see below).

B) PGI₂ AND PGE₁ ACTING ON ION CHANNELS *VIA* IP RECEPTORS

Whereas EP receptors have equal affinity to PGE₁ and PGE₂ (Fig. 6C), IP receptors have high binding affinity to PGI₂ as well as PGE₁, but not to PGE₂. Interaction of PGE₂ with rat IP receptors occurs only at PGE₂ concentrations approximately 300fold higher than its K_d for the rat EP2 and EP4 receptors (quoted from [90]). Comparison between PGE₁ and PGE₂ sensitivity can be used to distinguish between EP and IP receptors. In addition, two fairly selective IP agonists, cicaprost and the novel ONO-54918-07, are available. However, cicaprost in a concentration of 1 µM is likely to stimulate also EP3 receptors (also quoted from [90]). When studying the cardioprotective action of PGI₂ and cicaprost, stimulation of EP3 receptors by 10 µM cicaprost but not by 1 µM was observed [102].

According to [82], prostacyclin receptor mRNA is most abundantly expressed in neurons of the DRG and moderately in arterial smooth muscle cells of a variety of tissues. Likewise, neuroblastoma and neuroblastoma x glioma hybrid cells have a high density of IP receptors.

Arterial Smooth Muscle

PGI₂ and its stable analogue iloprost have a potent vasodilatory effect. At concentrations between 1 nM and 1 µM iloprost hyperpolarizes the smooth muscle cells of the canine carotid artery, presumably by opening K⁺ channels, and relaxes the tension of isolated carotid segments [103]. In smooth muscle cells of the rat tail artery, iloprost (0.5 µM) enhances the outward current through Ca²⁺-activated and ATP-sensitive K⁺ channels [96, 97].

DRG Cells and Other Neurons

PGI₂ like PGE₂ enhances the non-selective inward current elicited by capsaicin in mouse DRG cells [85]. The potentiating effect of PGI₂ and ONO-54918-07 on the capsaicin-induced current through TRPV1 channels was recently studied by Narumiya's group [76] and has been illustrated in Fig. 4E. Earlier work had been concerned with the effect of PGI₂ and IP agonists on K⁺ channels. On embryonic rat DRG cells, a time dependent inhibition of the delayed rectifier current not only by PGE₂ but also by the IP agonist carba prostacyclin (1 µM) was found [80], confirming the view that DRG cells possess both EP and IP receptors. In guinea pig nodose ganglion neurons, 0.1 µM prostacyclin abolishes the Ca²⁺-activated K⁺ current that gives rise to a slow afterhyperpolarization [114]. PGE₂ (0.3 µM) had no effect, but inhibited the slow afterhyperpolarization in rabbit nodose ganglion cells (see above).

At the rat isolated vagus nerve, PGI₂ in concentrations as low as 0.1-1 nM causes depolarization [105]. The rank order of agonist potency is PGI₂ = PGE₁ > PGE₂, consistent with IP receptor activation.

Neuroblastoma and Neuroblastoma x Glioma Hybrid Cells

In a comparison of selected activators of adenylate cyclase in the NCB-20 neuronal hybrid cell line, prostacyclin was observed to be 17 times more potent than PGE₁ and over 1000 times more potent than PGE₂ [8]. PGI₂ and PGE₁ compete for a single receptor in these cells [9]. Likewise, the receptor on mouse neuroblastoma x rat glioma hybrid NG108-15 cells for prostanoid agonists has been demonstrated to have the pharmacological profile of a

prostacyclin receptor (quoted from [2]). It binds PGE₁ with a K_D of 13-20 nM [69]. Cicaprost, the standard IP receptor agonist, potentially activates cAMP accumulation in F-11 (embryonic rat DRG x neuroblastoma hybrid), NG108-15 and human neuroblastoma SK-N-SH cells [105, 22].

In neuroblastoma cells neither a capsaicin-induced inward current nor a PGE₁ effect on the delayed rectifier have been found. PGE₁ and iloprost have, however, a pronounced effect on the leakage current and the Ca²⁺ currents of voltage-clamped NG108-15 cells (Figs 7 and 8). Voltage ramps (Fig. 7A and B) and rectangular pulses (Figs 7C and 8) are used. The current-voltage curves recorded with voltage ramps consist of a linear leakage current (between -70 and -45 mV), an inward current (between -45

Fig. (7). Effect of PGE₁ and iloprost on voltage clamp currents of NG108-15 cells. A and B: Current-voltage curves produced by voltage ramps in control and 3 μM PGE₁ (A) and in control, 5 nM iloprost and wash (B). Voltage ramps from -70 to 0 mV in 0.2 s (A) and in 1 s (B). C: T-type Ca²⁺ currents recorded with rectangular pulses to potentials between -40 and +10 mV in 10 mV steps in control, 0.2 μM PGE₁ and wash. Perforated patch with bath containing 2.6 mM Ca²⁺ in A and B, ruptured patch with bath containing 10 mM Ca²⁺ and K⁺-free pipette solution in C. Holding potential -70 mV. Temperature 21-25°C. From [70, 71] and Meves (unpublished). Curves in A and B fitted with a linear equation from -70 to -46 mV (A) or -50 mV (B) gave the following parameters:

	g [nS]	V _{rev} [mV]
A control	0.99	-31
PGE ₁	1.77	0
B control	0.70	-21
iloprost	3.22	-1
wash	0.80	-21

Fig. (8). Effect of PGE₁ and iloprost on the hva (=high voltage activated) Ca²⁺ current. A: Hva Ca²⁺ current at +15 mV in control and 0.2 μM PGE₁. B: Hva Ca²⁺ current at +15 mV in control and 50 nM iloprost. C: Peak current vs pulse potential from a different cell in control, 3 μM PGE₁ and wash. D: Same for current at end of 160 ms pulses. Ruptured patch with bath containing 10 mM Ca²⁺ and K⁺-free pipette solution. Holding potential -70 mV. Temperature 20-21°C. From [71].

and -10 mV) and a strong outward current (between -10 and 0 mV). The latter two currents represent T-type Ca²⁺ current and delayed rectifier, respectively. PGE₁ and iloprost have a clear and reversible effect on the leakage current and the T-type Ca²⁺ current whereas the delayed rectifier current is not affected. As seen in Fig. 7A, PGE₁ (3 μM) increases the conductance (g) of the leakage current (estimated from the slope of the current-voltage curve in its most negative voltage range), shifts the current's reversal potential V_{rev} (derived by extrapolating to $I=0$ the linear fitting of the I-V relationship) to more positive values and increases the inward current I_{hold} at the holding potential (-70 mV). The effect does not disappear when the NaCl in the bath is replaced by N-methyl-D-glucamine or TrisCl, indicating that PGE₁ opens channels with large pore diameters. Iloprost (5 nM) acts in a way similar to PGE₁ (Fig. 7B). PGE₁ also inhibits the T-type Ca²⁺ current. The inhibition of T-type Ca²⁺ current by PGE₁ is seen in Fig. 7A, but more clearly seen when rectangular clamp pulses are used (Fig. 7C). In a total of 39 experiments 0.2 μM PGE₁ inhibited T-type Ca²⁺ current (at pulse potentials between -15 and 0 mV) by $37.1 \pm 3.6\%$ (mean $\pm$ SEM). The inhibition by 50 nM iloprost was $26.2 \pm 5.7\%$ (n=9).

In further experiments the pulse potential range was extended in order to study the effect of PGE₁ and iloprost on hva (=high voltage activated) Ca²⁺ currents. Such currents are shown in Fig. 8A and B). Their time course and amplitude distinguish them clearly from the fast inactivating, smaller T-type Ca²⁺ currents. The inhibition of the hva Ca²⁺ currents by PGE₁ (0.2 μM) and iloprost (50 nM), visible in Fig. 8A and B), was stronger than the inhibition of the T-type Ca²⁺ currents measured on the same cell. Fig. 8C and D illustrate an experiment with 3 μM PGE₁. The current-voltage curves for the peak current and the current at pulse end show a conspicuous kink at +5 mV. Upon application of 3 μM PGE₁, the peak hva current in the entire potential range

from +15 to +45 mV was inhibited by 69-77% while the peak T-type current was only decreased by 36%. The difference is even more pronounced in Fig. 8D because hva current at the end of 160 ms pulses is not contaminated by T-type current.

The smallest PGE₁ concentration which in some cells produced an effect was 2.4 nM. The mean values for ΔI_{hold} and g_{PGE}/g_{contr} measured with 22 nM were similar to the mean values for 0.2 and 3 μM. Likewise, the curve for cAMP production versus [PGE₁] rises steeply to its maximum in the nanomolar range (see Fig. 17 of [38]). The same was true for iloprost. Mean values of ΔI_{hold} and $g_{iloprost}/g_{contr}$ were similar for 5, 50 and 200 nM (compare with curve cAMP production versus [iloprost] in Fig. 4 of [56]). The smallest effective iloprost concentration was 1 nM. The sensitivity to PGE₁ and iloprost varied greatly from cell to cell. This made it difficult to obtain proper dose-response curves. With increasing passage number cells often ceased to respond at all.

IP receptors couple to G_s and G_{q/11} and in some cases to G_i. They activate adenylate cyclase *via* G_s and phospholipase C (PLC) *via* G_{q/11}. Coupling to G_i, leading to inhibition of adenylate cyclase, is cell type-dependent for the mouse IP receptor and absent for the human IP receptor [22]. Thus, either increase of cAMP or [Ca²⁺]_i or activation of PKC is responsible for the effects of PGE₁, PGI₂ and its stable analogues (see below).

C) MECHANISM OF ACTION

EP Receptors Acting *via* cAMP-Dependent Protein Kinase A or *via* Protein Kinase C

Exposing sensory neurons to PGE₂ at concentrations that sensitize causes an increase in the production of cAMP. This has been first reported by Hingtgen, Waite and Vasko in 1995 [43] and then confirmed in many later publications.

Prostaglandin effects are often mediated by cAMP activated protein kinase A (PKA). Such prostaglandin effects are mimicked by forskolin or membrane-permeable cAMP analogues and blocked by compounds that inhibit adenylate cyclase or PKA. The best examples are the effects of PGE₂ on TTX-R Na⁺ channels and on K⁺ channels. They disappear when a peptide inhibitor of PKA or the powerful cAMP antagonist Rp-cAMPS, a phosphorothioate derivative of cAMP, are included in the pipette solution [26, 32, 27]. The observations on rat DRG cells were confirmed on TTX-R Na⁺ channels expressed in COS-7 cells, a cell line derived from African green monkey kidney [28]. The effect of forskolin vanished when all five PKA consensus phosphorylation sites within the intracellular I-II loop had been eliminated by site-directed mutagenesis (Fig. 9). Also, the enhancement of the capsaicin-induced inward current by PGE₂ (see Fig. 4A) is mimicked by forskolin and completely blocked by intracellular perfusion of the PKA inhibitor PKI₁₄₋₂₄ [64]. Sensitization of mechanosensitive channels by PGE₂ is blocked by the PKA inhibitor H-89, an isoquinoline sulfonamide [20]. On osteoclasts, forskolin or dibutyryl cAMP reproduce the stimulation of Cl⁻ current by PGE₂ whereas the cAMP antagonist Rp-cAMPS and the PKA inhibitor H-89 abolish it [83].

So far, the situation seemed straightforward. Further work showed, however, that it is in reality more complicated. First, it turned out that in addition to PKA the protein kinase C (PKC) plays an important role. Second, results were published which seem to suggest that under certain conditions neither PKA nor PKC are involved in the sensitizing effect of PGE₂.

It was found that augmentation of TTX-R I_{Na} by PGE₂ is attenuated not only by inhibitors of PKA (Rp-cAMPS or Wiptide) but also by the PKC inhibitors PKC₁₉₋₃₆ or staurosporine, "suggesting that PKC activity is necessary for PGE₂-induced modulation of TTX-R I_{Na}" [32]. A recent study [76] on adult mouse DRG cells and on HEK293 cells (expressing TRPV1 channels and either EP1 or EP4 receptors) has shown that potentiation of capsaicin-activated currents by PGE₂ (see Fig. 4C-E) involves both PKC-

dependent and PKA-dependent pathways. A short PGE₂ application (1.5 min) activates the pathway EP1 — PKC, a longer application (6.5 min) is required to stimulate the pathway EP4 — PKA. The effect of a short PGE₂ application is almost completely prevented by the specific PKC inhibitor calphostin C, blocked by the EP1 antagonist ONO-8713, significantly diminished by the PLC inhibitor U73122, mimicked by the EP1 agonist ONO-DI-004 and by the PKC activator PMA (phorbol 12-myristate 13-acetate). A mixture of forskolin, IBMX and dbcAMP, applied for 6.5 min, reproduces the effect of a long PGE₂ application.

PKA and PKC phosphorylate serine or threonine residues on ion channels, thereby modulating their function. Na⁺ channels have five sites of cAMP dependent phosphorylation (see Fig. 9), all situated on the large intracellular loop that connects domains I and II of the α subunit. The effects of phosphorylation on Na⁺ channels are fully discussed in a recent review [18]. Two target serine residues are found in the capsaicin receptor TRPV1 expressed in HEK293 cells [81]. They are phosphorylated by PKC ϵ and responsible for the potentiation of capsaicin-evoked currents by the phorbol ester PMA.

Two studies did not confirm the view that the potentiating PGE₂ effect on capsaicin-induced current and TTX-R Na⁺ current is mediated by PKA and PKC. In the experiments of Lee, Lee, Jung, Oh and Kaang [62], activation of PKA by 8-Br-cAMP or forskolin with IBMX failed to enhance capsaicin-evoked inward currents in *Xenopus* oocytes expressing TRPV1 channels. A recent study on neonatal rat nodose ganglion neurons suggested that the increase in TTX-R I_{Na} induced by PGE₂ application may not involve the activation of either PKA or PKC activity [67]. In these experiments, bath application of 1 μ M PGE₂ produced the well known increase of TTX-R I_{Na} together with a leftward shift of the Na⁺ conductance curve g_{Na}(V). A subsequent intracellular application of the PKA inhibitor PKI (in the continuing presence of 1 μ M PGE₂) decreased I_{Na} again, but did not reverse the shift of the g_{Na}(V) curve. Likewise, staurosporine reversed the PGE₂ effect on the peak amplitude of I_{Na}, but had no significant effect on the

Fig. (9). Effect of 10 μ M forskolin on TTX-R I_{Na} expressed in COS-7 cells. A: Wild-type SNS. B: Mutant SNS (SA) with all five PKA consensus sites eliminated. Peak Na⁺ currents (elicited by stepping from -90 to +15 mV (A) or +20 mV (B)) are plotted against time. Currents recorded at times 1 and 2 are shown in insets. From [28].

hyperpolarizing shift of $g_{Na}(V)$. Obviously, it depends very much on the experimental conditions whether evidence for the involvement of PKA and PKC is obtained. Further work must clarify the problems.

EP Receptors Acting *via* Increase of Intracellular Calcium

Clearly, some of the PGE₂ effects are caused by an increase of intracellular calcium. As mentioned above, PGE₂ acting on EP1 and EP3 receptors can increase $[Ca^{2+}]_i$. On CHO cells stably expressing the mouse EP1 receptor the increase in $[Ca^{2+}]_i$ elicited by the EP1 agonist sulprostone consists of two parts [54]. Most of it is caused by Ca^{2+} influx from the bath, a small part by Ca^{2+} release from an internal store; the latter is sensitive to the PLC inhibitor U73122 and mediated *via* $G_{q/11}$ [109]. The increases in intracellular calcium elicited by PGE₂ and various other agonists in HEK293 cells expressing apo-aequorin and the rat EP1 receptor have been measured [12]. The elevation of cytosolic calcium by PGE₂ in neonatal rat DRG neurons has been studied [106]. PGE₂ at 1 μ M – in addition to increasing cAMP – evoked an increase of $[Ca^{2+}]_i$ in 50% of the small DRG neurons. Pretreatment with H-89 reduced the number of cells that responded to 24%. The Ca^{2+} signal disappeared in Ca^{2+} -free bath containing 0.2 mM EGTA and was not accompanied by an increase of IP₃ levels, suggesting that it is caused by Ca^{2+} influx from the bath. This may occur either through voltage-gated calcium channels (as suggested by the authors) or through non-selective cation channels. Also on rat DRG neurons, the PGE₂-induced Ca^{2+} -influx is blocked by the non-selective calcium channel blocker Cd²⁺ [63]. The membrane permeant cAMP analogue dibutyryl cAMP acted similarly to PGE₂. An increase of $[Ca^{2+}]_i$ due to Ca^{2+} influx is most likely the reason for the activation of Ca^{2+} -activated K⁺ channels in human osteoblast bone cells by PGE₂ [75]. The effect is mimicked by cAMP + forskolin and disappears in Ca^{2+} -free bath. An increase of $[Ca^{2+}]_i$ could also be responsible for the inhibition of Ca^{2+} currents by 1 μ M PGE₂ in rat sympathetic neurons [45] illustrated in Fig. 6A and B. Inhibition became smaller when the pipette solution contained 10 mM BAPTA instead of EGTA.

cAMP Acting Directly on I_h Channels

As mentioned above, PGE₂ increases the hyperpolarization-activated current I_h in some guinea-pig neurons [46]. The effect consists of a shift of the I_h activation curve to more positive potentials and is mimicked by forskolin. The PKA inhibitor PKI does not affect the activation curve and does not block the effect of forskolin, suggesting modulation of I_h through direct action of cAMP on the channel proteins. An enhancement of I_h by a PKA-independent, direct action of cAMP was also demonstrated in I_h channels of hippocampal pyramidal cells [84] and in heterologously expressed hyperpolarization-activated channels [65]. By contrast, a PKA-dependent regulation by cAMP was found in bullfrog sympathetic neurons [112] and more recently in rat olfactory receptor neurons [115]. Experiments on mouse DRG cells [86] suggest two separate processes responsible for the enhancement of I_h, a direct effect and, in addition, phosphorylation through PKA. Conflicting results were also obtained for the hyperpolarization-activated current in

cardiac tissue. A direct cAMP effect was observed by DiFrancesco's group (see [13]) whereas another study [125] reported an important role of phosphorylation *via* cAMP-dependent PKA. Obviously, phosphorylation-dependent modulation of I_h varies from cell type to cell type, a situation similar to that described above for the PGE₂ effect on the capsaicin-elicited current and on TTX-R I_{Na}.

IP Receptors Coupling to Adenylate Cyclase and Phospholipase C

In the simplest case an IP agonist acts solely through elevation of intracellular cAMP. This is true for the iloprost-induced inward Cl⁻ current observed in *Xenopus* oocytes coexpressing the human IP receptor and the cystic fibrosis transmembrane conductance regulator [11]. In general, however, the situation is more complicated because IP receptors not only couple to adenylate cyclase but also to PLC, i.e. produce inositol phosphates and $[Ca^{2+}]_i$ increase in addition to cAMP (for review of the older literature, see [121]). The EC₅₀ values for coupling to PLC are higher than those for cAMP production, especially in transfected cells. For the human prostacyclin receptor expressed in HEK293 cells and activated by iloprost EC₅₀ = 0.1 and 43.1 nM for cAMP and inositol phosphate production, respectively, were obtained [107]. On CHO cells expressing the mouse prostacyclin receptor, PLC is stimulated with 10-40 fold lower potency than adenylate cyclase [21] (Fig. 10A and B). Accumulation of cAMP and [³H]-inositol phosphates in adult rat DRG cells treated with PGI₂, cicaprost or iloprost has been described [105]. In these cells, cicaprost and iloprost stimulate adenylate cyclase activity with EC₅₀ values of 22 and 28 nM, respectively [90]. Detailed quantitative studies were carried out on neuroblastoma cells (N1E-115) and neuroblastoma x glioma hybrid cells (NG108-15). As described above, these cells endogenously express IP receptors. Application of PGE₁ causes a marked increase of cAMP, reaching a plateau after 10-15 min [39, 99]. The half maximal effect is seen at 20 nM in Fig. 17 of [38] and at 70 nM in Fig. 10C. Iloprost is an even more potent agonist. According to [119] the EC₅₀ for iloprost is 24 nM, much lower than the average of ca. 100 nM for PGE₁ estimated from the results of different authors. PGE₂ is less effective than PGI₂ and PGE₁ (see Fig. 2B of [101], Fig. 3 of [42] and Table 1 of [50]). As illustrated in Fig. 10C, PGE₁ also causes an increase of inositol phosphates, cGMP and cytosolic Ca^{2+} in N1E-115 cells, but the EC₅₀ values (1-5 μ M) are more than 10 times higher than the EC₅₀ for cAMP production (70 nM). More recently, Chow, Jones and Wise [22] studied the effect of cicaprost on [³H]-cAMP and $G_{q/11}$ -mediated [³H]-inositol phosphate production in NG108-15 cells and human neuroblastoma SK-N-SH cells as well as CHO and HEK293 cells expressing human or mouse IP receptors. Cicaprost activated PLC in all cells except the SK-N-SH cells, but the EC₅₀ (49-457 nM) was much higher than the EC₅₀ for cAMP production (1.5-22 nM).

In the case of adipose cells, the PGI₂-induced transient Ca^{2+} accumulation does not appear to depend on extracellular calcium, i.e. is due to release from an internal store [116]. In erythroleukemia cells, cicaprost and iloprost increase $[Ca^{2+}]_i$ by mobilization from internal stores and

Fig. (10). Coupling of IP receptors to adenylate cyclase and phospholipase C. A: Production of [3 H]-cAMP and [3 H]-inositol phosphates in CHO cells transfected with mIP plotted against cicaprost concentration. B: Same for PGE₁. C: Production of cAMP, cGMP, inositol phosphates and cytosolic calcium in neuroblastoma cells N1E-115 plotted against PGE₁ concentration. The levels of cAMP, cGMP and inositol phosphates were measured after 20, 15 and 45 min incubation with the indicated PGE₁ concentrations. The values for [Ca^{2+}]_i give the maximum reached at 10–20 s. A and B from [21], C from [50].

influx from the extracellular space [98]. In other preparations Ca^{2+} entry from the bath prevails. Thus, the PGI₂-induced increase of [Ca^{2+}]_i in rat DRG cells disappears in Ca^{2+} -free bath [106]. According to [50] and [73] the increase of [Ca^{2+}]_i produced by PGE₁ and other prostaglandins in neuroblastoma cells also depends on the presence of Ca^{2+} in the extracellular solution, i.e. reflects Ca^{2+} entry. It is important to notice that the increase of [Ca^{2+}]_i is only transient, reaching a peak after 10–20 s and returning to the base line within 2 min (see Fig. 3 of [73]), whereas the cAMP level is sustained for minutes. Fig. 10C gives the peak values for [Ca^{2+}]_i, but values for cAMP, cGMP and inositol phosphates determined after 15–45 min incubation (see figure legend).

The PGI₂ effects on arterial smooth muscle and TRPV1 channels are mediated by PKA and PKC. The enhancement

of outward currents through Ca^{2+} -activated K^{+} channels by 0.5 μM iloprost in arterial smooth muscle (see above) appears to be caused by PKA phosphorylating the channels. The iloprost effect is mimicked by the catalytic subunit of PKA, applied *via* pipette together with 0.1 mM MgATP [96]. By contrast, the potentiation of the capsaicin-elicited inward current by PGI₂ (see Fig. 4E) in mouse DRG neurons and HEK293 cells expressing TRPV1 channels and IP receptors involves both PKC and PKA [76]. The situation is similar to that described above for the PGE₂ effect. A short application (1.5 min) of a fairly high PGI₂ concentration (1 μM) activates predominantly the pathway $\text{IP} \rightarrow \text{G}_q \rightarrow \text{PKC}$, a long (6.5 min) treatment with 0.1 μM PGI₂ stimulates the pathway $\text{IP} \rightarrow \text{G}_s \rightarrow \text{PKA}$. Blockers of PKC and PLC prevent only the effect of a short (1.5 min) PGI₂ application.

Effects of PGE₁ on Ionic Currents of Neuroblastoma Cells

The effects of PGE₁ and iloprost on ionic currents of NG108-15 cells (Fig. 7 and 8) are caused by an increase of both cAMP and [Ca^{2+}]_i. Experiments that support this view are shown in Fig. 11. An increase of holding current like that seen with PGE₁ and iloprost is produced by the membrane permeable cAMP analogue CPT-cAMP (Fig. 11A), the adenylate cyclase activator forskolin and thapsigargin (Fig. 11B). The latter substance releases Ca^{2+} from internal stores and produces in NG108-15 and N1E-115 cells an immediate

Fig. (11). Experiments supporting the view that PGE₁ acts on NG108-15 cells *via* increase of intracellular cAMP and Ca^{2+} . A–C: Pen records of holding current recorded at -70 mV. A: Effect of 0.5 mM CPT-cAMP. B: Effect of 1 μM thapsigargin. C: Effect of 0.2 μM PGE₁ in Ca^{2+} - and Mg^{2+} -free bath with 1.1 mM EGTA. D: Scatter plot showing the effect of 3 μM PGE₁ on holding current with 3 mM EGTA ($n=18$) or 20 mM BAPTA ($n=7$) in pipette solution; mean values indicated by horizontal bars. Perforated patch in A–C, ruptured patch in D. Room temperature. From [70, 71] and Meves (unpublished).

increase of [Ca^{2+}]_i by 150–200 nM [88, 66]. On average, the change in holding current was -151.5 ± 27.3 pA ($n=5$) in 0.5 mM CPT-cAMP [70] and -67.5 ± 17.9 pA ($n=5$) in 1 μM thapsigargin [71]. Together with the increase in holding current the current-voltage curves measured with voltage ramps become steeper between -70 and -50 mV, indicating an increase in conductance. In Ca^{2+} - and Mg^{2+} -free bath

solution with 1.1 mM EGTA, 0.2 μM PGE₁ could still produce a clear change in holding current. It was -125 pA in Fig. 11C and on average $-103.1 \pm 28.1 \text{ pA}$ ($n=8$) [71]. As shown by the scatter plot in Fig. 11D, the PGE₁ effect is, however, markedly reduced when the pipette solution contains 20 mM of the fast acting chelating agent BAPTA instead of the usual 3 mM EGTA. Likewise, the inhibition of the T-type Ca^{2+} current by 0.2 μM PGE₁ (see Fig. 7C) becomes smaller; it is $37.1 \pm 3.6\%$ ($n=39$) with 5 mM EGTA in the pipette solution and only $16.0 \pm 2.5\%$ ($n=5$) with 5 mM BAPTA. In conclusion, the experiments of Fig. 11 suggest that the PGE₁ effect is caused by an increase of internal cAMP and by release of Ca^{2+} from intracellular stores. In addition, the transient Ca^{2+} entry from the bath may play a role. It may be responsible for the large increase of holding current seen in many cells (e.g. effect of 50 nM iloprost in Fig. 1C of [71]).

The change in holding current was significantly reduced by flufenamic acid (FFA), an inhibitor of Ca^{2+} -activated non-selective cation current [30, 37]. With 0.1–0.9 mM FFA in the bath, the increase in holding current produced by 3 μM PGE₁ was only $-32.2 \pm 7.7 \text{ pA}$ ($n=9$) compared with $-106.7 \pm 23.3 \text{ pA}$ ($n=16$) in control cells of the same batch.

In rat DRG cells, the membrane permeant dibutylryl cAMP causes Ca^{2+} entry and increase of $[\text{Ca}^{2+}]_i$ [106]. In N1E-115 cells, the adenylate cyclase activator forskolin increases $[\text{Ca}^{2+}]_i$ by activation of PLC- ϵ [95]. Thus, it may be thought that the first step in the PGE₁ effect on neuroblastoma cells is a rise in cAMP which then increases $[\text{Ca}^{2+}]_i$ by a dual mechanism, opening of non-selective cation channels and release from internal store. Increase of intracellular Ca^{2+} would cause a further opening of non-selective cation channels (as described in [124]), thus permitting further Ca^{2+} to enter. This regenerative process would explain the strong conductance increase and the concomitant blockage of Ca^{2+} currents observed upon application of PGE₁ or iloprost. A synergistic action of intracellular Ca^{2+} and cAMP is also thought to occur in pancreatic β cells where it is responsible for the initiation of Ca^{2+} -induced Ca^{2+} release [52].

Some support for the assumed key role of cAMP comes from the observation that cAMP production and effect on holding current both start at the same PGE₁ concentration, namely at about 10 nM [70]. Rp-cAMPS almost totally abolishes the effect of 0.2 and 3 μM PGE₁ on holding current, when applied *via* patch pipette [71]. By contrast, the PKA inhibitor H-89 which was applied *via* bath in the fairly high concentration of 10–30 μM or *via* pipette, was ineffective. It did not prevent the PGE₁ effect on holding current and T-type Ca^{2+} current. Fig. 12A shows nearly total blockage of the T-type I_{Ca} by 0.2 μM PGE₁ in a cell pretreated with bath containing 10 μM H-89 for 88 min. Likewise, PKC₁₉₋₃₁, a peptide inhibitor of protein kinase C, did not inhibit the PGE₁ effect on T-type I_{Ca} .

The mechanism by which PGE₁ and forskolin produce an increase of $[\text{Ca}^{2+}]_i$ in N1E-115 cells has been analyzed in detail [95, 55]. According to this study, cAMP acts *via* Epac (=exchange protein activated by cAMP) on PLC- ϵ which in turn produces inositol phosphates and an increase of $[\text{Ca}^{2+}]_i$.

Fig. (12). The PKA inhibitor H-89 does not prevent blockage of T-type Ca^{2+} current by PGE₁ in NG108-15 cells, but the Epac agonist 8-pCPT-2Me-cAMP mimics the PGE₁ effect on holding current and current-voltage curve. A: T-type Ca^{2+} currents at -25 and -15 mV in control, in 0.2 μM PGE₁ (applied for 3 min) and after 4 min wash. When PGE₁ was applied, the cell had been in bath with 10 mM CaCl_2 and 10 μM H-89 for 88 min. Temperature 26.5°C. B and C: Effect of 300 μM 8-pCPT-2Me-cAMP on the holding current (B) and on the current-voltage curve (C). Asterisks in B indicate current-voltage curves shown in C. Temperature 22.8°C. From [71] and Meves (unpublished).

PKA is not involved in the signaling path. Experiments illustrated in Fig. 12B and C show that the Epac agonist 8-pCPT-2Me-cAMP can indeed mimic the PGE₁ effect on holding current and current-voltage curve of NG108-15 cells. However, signaling *via* Epac and PLC- ϵ cannot be the only mechanism responsible for the PGE₁ effect on NG108-15 cells. As shown in [71], the PGE₁ effect on NG108-15 cells is not significantly reduced by the aminosteroid U73122, a powerful PLC inhibitor, but totally inhibited by the cAMP antagonist Rp-cAMPS (for which Epac is said to have a low affinity, see [51]). Very likely, there are cAMP-dependent events independent of either PKA or Epac.

The diagram in Fig. 13 depicts the various pathways so far known to be involved in the effects of cAMP. It emphasizes the fact that cAMP alters the intracellular Ca^{2+} concentration which in turn affects $[\text{cAMP}]_i$.

Fig. (13). Diagram summarizing the known effects of cAMP and illustrating the interplay of cAMP and Ca^{2+} signaling. For details of cAMP – Ca^{2+} interplay see [16, 126].

The mechanism of action is, in general, similar for EP- and IP-mediated prostaglandin effects. In both cases it includes a rise of the intracellular level of cAMP and Ca^{2+} and, in some preparations, activation of protein kinases A and C. The effects are also similar: Sensitization of nerve cells, potentiation of the capsaicin-elicited inward current, inhibition of K^{+} and Ca^{2+} currents. Kwong & Lee [60] have drawn attention to one remaining open question: Whether PGI_2 and PGE_1 , acting *via* IP receptors, enhance the TTX-resistant Na^{+} current, has yet to be determined.

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