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ATP-Sensitive Potassium Currents Reduce the PGE₂-Mediated Enhancement of Excitability in Adult Rat Sensory Neurons

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Abstract

Behavioral studies have shown that the hyperalgesia arising from inflammatory agents, such as prostaglandin E₂ (PGE₂) can be antagonized by activators of the ATP-sensitive potassium current (K_{ATP}). This observation raises questions as to whether this suppression results from a direct action on sensory neurons and what are the cellular mechanisms giving rise to this inhibition. We found that small to medium diameter sensory neurons isolated from the L4-6 DRGs expressed the mRNAs for Kir6.1, Kir6.2, and SUR1. In perforated-patch clamp recordings from acutely dissociated sensory neurons from the young adult rat, exposure to 300 μM diazoxide, a K_{ATP} channel agonist, significantly hyperpolarized the resting membrane potential, reduced the number of action potentials evoked by a ramp of depolarizing current, and increased the amplitude of inward K_{ATP} currents evoked by the voltage ramp. Similar results were obtained with the protonophore FCCP, which is known to reduce the levels of intracellular ATP and lead to the activation of K_{ATP}. Only a subpopulation of sensory neurons was sensitive to diazoxide whereas other neurons were unaffected. Treatment with 1 μM PGE₂ significantly enhanced the excitability of these small to medium diameter capsaicin-sensitive sensory neurons; this enhancement was reversed by subsequent exposure to diazoxide in a subpopulation of neurons. Similar to diazoxide, exposure to 8-Br-cyclic GMP antagonized the PGE₂-induced increase in excitability. The effects of 8-Br-cyclic GMP could be reversed by exposure to glibenclamide, an antagonist of K_{ATP} channels. As with diazoxide, only a subpopulation of sensory neurons were affected by 8-Br-cyclic GMP. These results demonstrate that activation of K_{ATP} can reverse the sensitization produced by PGE₂ and may be an important means to modulate the enhanced excitability that results from inflammatory or injury conditions.

Keywords

K_{ATP}; diazoxide; cyclic GMP; sensitization; action potential

1. Introduction

The ATP-sensitive potassium channels (K_{ATP}) belong to a family of inwardly rectifying potassium channels. K_{ATP} channels, originally identified in cardiac myocytes (Noma 1983), are also present in the central nervous system (Amoroso et al. 1990; Murphy and Greenfield

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1991;Li and Henry 1992;Ohno-Shosaku and Yamamoto 1992). The K_{ATP} channel is an octomeric complex composed of either the Kir6.1 or Kir6.2 channel subunits and sulphonylurea regulatory subunits (SUR) (Inagaki et al. 1996;Aguilar-Bryan et al. 1998;Ashcroft and Gribble 1998;Babenko et al. 1998). K_{ATP} channels can be inhibited by either high or physiological concentrations of intracellular ATP, therefore, decreasing levels of intracellular ATP can result in activation of K_{ATP} (Ammala et al. 1996;Aguilar-Bryan et al. 1998;Trapp et al. 2003). Because these channels are regulated by the levels of ATP, they provide an important link between cellular metabolism and excitability (Takei et al. 1985;Takei et al. 1986;Ashcroft 1988;Ashcroft and Takei 1989). In this regard, K_{ATP} channels control the release of insulin from pancreatic β cells (Ashcroft et al. 1984;Ashcroft and Gribble 1998;Miki et al. 1999;Schwanstecher et al. 1998;Seino 1999) and blood flow in muscle, heart, and kidney tissue (Ashcroft 1988;Nelson and Quayle 1995;Terzic et al. 1995;Yokoshiki et al. 1998). In neurons, activation of K_{ATP} results in membrane hyperpolarization that reduces excitability (Yamada et al. 2001;Allen and Brown 2004) and activation of presynaptic K_{ATP} channels can directly modulate neurotransmitter release from nerve terminals (Deist et al. 1992;Ohno-Shosaku et al. 1992;Watts et al. 1995;Ye et al. 1997).

Based on the capacity of K_{ATP} to reduce neuronal excitability, a putative link between K_{ATP} and the action of several antinociceptive agents was examined. Early work indicated that K_{ATP} may mediate the analgesic effects of morphine (Ocana et al. 1990) or clonidine (Ocana et al. 1993) since the antinociceptive effects of these agents could be reversed by pretreatment with glibenclamide, a selective K_{ATP} antagonist. Recent studies indicate that glibenclamide can antagonize the antinociceptive effects of a variety of compounds such as nitric oxide and cyclic GMP in an assortment of animal models of nociception, such as those wherein hyperalgesia is produced by carrageenan or prostaglandin E_2 (PGE_2) (Rodrigues and Duarte 2000;Soares et al. 2001;Alves and Duarte, 2002). Interestingly, other potassium channels blockers such as tetraethylammonium or 4-aminopyridine had no effect on the antinociception. Several of these reports indicate a linkage between the cyclic GMP signalling pathway and activation of K_{ATP} (Lazaro-Ibanez et al. 2001;Soares and Duarte 2001;Sachs et al., 2004). Indeed, studies of other cellular systems have demonstrated that cyclic GMP can lead to the activation of K_{ATP} (Kubo et al. 1994;Murphy and Brayden, 1995;Armstead 1997, 1998;Schwingshackl et al. 2002). These behavioral measures of the antinociceptive actions of K_{ATP} suggest that this channel may play an important role in reversing or antagonizing the sensitizing effects of a variety of inflammatory mediators. However, behavioral studies provide little evidence as to whether K_{ATP} channels are localized to nociceptive sensory neurons or other associated cell types such as glia or support cells. In electrophysiological studies, Sarantopoulos et al. (2003) found that pinacidil, but not diazoxide, which are agonists of K_{ATP} , enhanced the currents evoked by depolarizing voltage steps in rat DRG neurons. Their findings suggested that K_{ATP} channels are localized in sensory neurons. Also, such studies cannot examine the direct cellular mechanisms of action or the intracellular signalling pathways modulating K_{ATP} activity. Therefore, the aim of the present study was to examine whether gene transcripts for subunits of the Kir6 and SUR families were expressed in sensory neurons isolated from the adult rat. In addition, the capacity of K_{ATP} and the cGMP signalling pathway to reverse the sensitization produced by the pro-inflammatory PGE_2 was determined.

2. Results

Small to medium diameter sensory neurons express mRNA for Kir6.1/Kir6.2 and SUR1

To determine whether small diameter sensory neurons of the L4-6 DRG contained the mRNAs for the subunits of SUR, RT-PCR was used to detect these gene transcripts under optimal conditions established specifically for these primers. Figure 1A illustrates the results obtained from an experiment using total cDNA obtained from the intact ganglion preparation wherein

both SUR1 (S1, product size 181 bp) and SUR2 (S2, targeted to both SUR2A and 2B, product size 144 bp) were detected at their expected sizes and indicate that these primers were effective in detecting their designated targets. Similar results were obtained from another intact ganglion preparation where both SUR1 (SUR1 lane 2, Fig. 1B) and a faint band for SUR2 (SUR2 lane 2, Fig. 1B) were detected. To ascertain whether the mRNA for the SURs was specific to the neuronal population, samples were obtained from two separate groups of five small to medium diameter sensory neurons (<35 μ m in diameter). This value for the neuronal diameter was based on the observations of Harper and Lawson (1985) which demonstrated that the small-dark neurons or neurons conducting at C-fiber velocities had average diameters of approximately 24 ± 13 μ m (mean $\pm$ SD). RT-PCR indicated that the mRNA for SUR1 was detected (see Fig. 1B, SUR1 lanes 1 and 3). In contrast, the primer for SUR2 failed to detect SUR2 mRNA in a sample from five small to medium diameter sensory neurons (SUR2 lane 1) even though these same neurons expressed SUR1 (the same cDNA was used for SUR1 lane 3 and SUR2 lane 1). Furthermore, the mRNA for SUR1 was detected in two of five small to medium diameter sensory neurons using single-cell RT-PCR (see Fig. 1C, +RT lanes 3-7) and is consistent with the electrophysiological recordings described below. As a negative control, no PCR products were detected for either the blank/no template controls (Fig. 1, lanes labelled with B) or samples that were processed in the absence of reverse transcriptase (Fig. 1C, -RT, lanes 1-5). For positive confirmation, SUR1 was detected using total cDNA obtained from the intact ganglion (Fig. 1C +RT lanes 1 and 2), and served as negative controls that demonstrate these PCR products did not result from genomic contamination. To determine which K_{ATP} channels were potentially associated with the SUR, RT-PCR was used to detect the mRNAs for Kir6.1 and Kir6.2 under optimal conditions established specifically for these primers. Figure 2 shows a representative gel where bands for Kir6.1 and Kir6.2 were observed at their expected product sizes (247 bp for Kir6.1, 167 bp for Kir6.2). In the total cDNA obtained from the same intact ganglia preparation (lanes labeled total in left and right panels) Kir6.1 and Kir6.2 were detected. This, however, does not establish whether Kir6.1 and Kir6.2 are expressed in small to medium diameter sensory neurons. To address this question, single cell RT-PCR was used wherein the mRNA for Kir6.1 and Kir6.2 were detected in the same individual small diameter DRG neurons (20-25 μ m, individual cells labeled S1, S2, S3, S4 and S5). There was no mRNA detected for Kir6.1 or Kir6.2 in cell S1. The mRNA for both Kir6.1 and Kir6.2 were detected in cells S2, S3, and S5 (although detectable, Kir6.2 in S2 and S5 were very faint). In cell S4, Kir6.1 but not Kir 6.2 was detected. No PCR products were detected in the no template control (lanes B). Thus, these results demonstrate that small to medium diameter sensory neurons isolated from young adult rats expressed the mRNAs for the K_{ATP} channel subtypes Kir6.1 and Kir6.2, which are likely associated with SUR1. The specific localization of the subtypes of SUR2 remains an area of future investigation.

K_{ATP} and excitability in sensory neurons

Based on the expression of gene transcripts for Kir6.1/Kir6.2 and SUR1, we sought to establish whether a K_{ATP} channel opener could alter the excitability of small to medium diameter capsaicin-sensitive sensory neurons. The current clamp configuration was used to examine the effects of diazoxide on the capacity of sensory neurons to fire APs in response to a ramp of depolarizing current. As shown in a representative neuron (see Fig. 3A), a 5 min exposure to 300 μ M diazoxide hyperpolarized the resting membrane potential by 5 mV and decreased the number of APs evoked by the current ramp. Note that the voltage at which the AP appears to fire was more depolarized after exposure to diazoxide. The results for the current clamp experiments obtained from a total of seventeen sensory neurons are summarized in Fig. 3B. Based on the capacity of diazoxide to reduce the number of APs, these sensory neurons could be segregated into two groups, one defined as sensitive to diazoxide group wherein the number of APs evoked by the current ramp was reduced by diazoxide (8 of 17), and one that was insensitive to diazoxide wherein the number of APs was unaffected by diazoxide (9 of 17).

The top panel of Fig. 3B illustrates in those sensitive neurons, the ability of diazoxide to significantly reduce the number of APs from an average control value of 8.4 ± 1.2 to 2.3 ± 0.7 APs (RM ANOVA). It should be noted that after this 5 min treatment, 3 of the 8 neurons no longer fired APs in response to the ramp. The inhibitory effects of diazoxide were reversible whereafter a 5 min washout period, the number of APs returned to control values (8.7 ± 1.2 APs, $n=8$). All three neurons that did not fire APs in the presence of diazoxide returned to their control values after washout. The decrease in AP firing was accompanied by a significant hyperpolarization of the resting membrane potential (Fig. 3B, middle panel) and a significant two fold increase in the rheobase when compared to the control level (Fig. 3B, bottom panel). All of these observations are indicative of reduced excitability. In contrast, neither the resting membrane potential, the rheobase, nor the number of APs was altered in those nine neurons judged to be insensitive to diazoxide (summarized in Fig. 3B).

Previously, Gribble et al. (1997) showed that FCCP, which is a protonophore that uncouples the mitochondrial transport chain from ATP synthesis, lowered the level of intracellular ATP in *Xenopus* oocytes and this decrease in ATP lead to activation of Kir6.2. In a separate series of current clamp experiments, the effects of FCCP on neuronal excitability were examined. In those neurons judged to be sensitive, a 10 min exposure to $5 \mu\text{M}$ FCCP significantly reduced the number of APs from a control value of 4.7 ± 0.8 to 1.5 ± 0.5 ($n=11$) and is similar to that observed for diazoxide. In three neurons that were insensitive, a 10 min application with FCCP did not significantly alter the number of APs (control 7.0 ± 0.6 compared to 6.0 ± 1.5 after FCCP). These results indicate that activation of K_{ATP} channels can suppress the excitability of a specific group of small to medium diameter capsaicin-sensitive sensory neurons.

K_{ATP} current in sensory neurons

To assess the K_{ATP} current, a voltage ramp was used to measure the change in current amplitude in the absence or presence of diazoxide. As shown in a representative current trace (Fig. 4A, left panel), a 5 min exposure to $300 \mu\text{M}$ diazoxide enhanced the amplitude of the current evoked by the voltage ramp. The current amplitude measured at -110 mV increased from a control value of -294 pA to -589 pA after exposure to diazoxide. This enhancement of the current by diazoxide is shown at greater resolution in the right panel. The value of the current measured at -110 mV was used to assess the effects of diazoxide; these results are summarized in panel B. A neuron was judged to be sensitive to diazoxide if the current was increased by $>10\%$ of the control value at -110 mV . After a 5 min exposure to diazoxide, the current was increased by approximately two fold as measured in nine neurons that were judged to be sensitive. The increased current amplitude persisted throughout the exposure to the agonist. In contrast, diazoxide had no effect in the six neurons that were judged to be insensitive ($<10\%$ change in the current at -110 mV). Panel C illustrates the results obtained with diazoxide for these same neurons when the current values have been normalized to their respective control values measured at -110 mV . This finding is consistent with the above observation that diazoxide hyperpolarized the resting membrane potential, but in only a specific population of neurons.

Activation of K_{ATP} reverses the sensitization produced by prostaglandin E_2

Behavioral studies examining mechanical hyperalgesia have suggested an antinociceptive role for K_{ATP} (see Introduction). Based on these observations, we determined whether activation of K_{ATP} might decrease the elevated excitability or sensitization produced by the pro-inflammatory prostaglandin, PGE_2 . Previous reports have shown that, in isolated sensory neurons, treatment with PGE_2 increased the number of APs evoked by either chemical excitation or a depolarizing current (Baccaglini and Hogan 1983; Nicol and Cui 1994; Gold et al. 1996b; Nicol et al. 1997). As shown in Fig. 5A, under control conditions (top left panel), a representative neuron fired 5 APs in response to a ramp of depolarizing current. However, after a 5 min exposure to $1 \mu\text{M}$ PGE_2 (top right panel), this neuron now fired 12 APs, indicating

that the excitability was enhanced by PGE₂. In the presence of PGE₂, a 5 min application of 300 μ M diazoxide decreased the number of APs to 7 (bottom left panel). The suppressive effects of diazoxide appeared to be reversible because after a 5 min washout of both diazoxide and PGE₂ (bottom right panel), the number of APs returned to 10. As described above, the neurons could be grouped into two subpopulations. One group was sensitive to diazoxide where, in the presence of PGE₂, diazoxide reduced the number of evoked APs (11 of 17 total, different neurons than shown in Fig. 3) and another that was insensitive to diazoxide where the PGE₂-induced increase in the number of APs was unaltered (6 of 17 total). The results obtained from both subtypes of neurons are summarized in Fig. 5B. In those neurons judged to be diazoxide sensitive, a 5 min treatment with PGE₂ significantly increased the number of APs from a control value of 5.9 ± 0.7 to 15.2 ± 1.5 APs ($n=11$, RM ANOVA). The addition of diazoxide to those neurons sensitized by PGE₂ produced a significant decrease in the number of evoked APs from 15 to 9.5 ± 1.7 APs (RM ANOVA). The suppressive effects of diazoxide appeared to be reversible as washout returned the number of APs towards that observed with PGE₂ alone. These results indicate that the actions of diazoxide are readily reversible although the sensitizing effects of PGE₂ may not be within the time frame of this experiment. In the other group of sensory neurons, diazoxide had no effect on the enhanced excitability produced by PGE₂ (see Fig. 5B). Because of the cell to cell variability in the number of evoked APs, the number of APs fired under the different experimental treatments was normalized to the number of APs recorded under their respective control conditions. These results are illustrated in Fig. 5C wherein exposure to diazoxide in the sensitive group significantly decreased the number of APs evoked in the presence of PGE₂ whereas diazoxide could not reverse the sensitization in the insensitive group. Thus, these results demonstrate that PGE₂ augments the excitability of small to medium diameter capsaicin-sensitive sensory neurons and that diazoxide is capable of antagonizing this enhanced excitability in only in a subgroup of neurons whereas the diazoxide-insensitive subgroup remains sensitized.

In both diazoxide-sensitive and -insensitive neurons (see Fig. 6 top panel), treatment with PGE₂ significantly decreased the rheobase (the value of the ramp current measured at the firing threshold) by about 50% when compared to the control values. These results indicate that PGE₂ enhanced the excitability of these neurons. In those neurons judged to be diazoxide-sensitive, PGE₂ decreased the rheobase from a control value of 1735 ± 358 to 792 ± 168 pA ($n=9$, RM ANOVA) and corresponds to a $52 \pm 5\%$ reduction. After treatment with diazoxide, the value of the rheobase increased to 1523 ± 388 pA ($n=9$) and was not different from the control value. These results indicate that diazoxide reversed the enhanced excitability produced by PGE₂. Two of the eleven neurons represented in Fig. 5 did not fire APs after the diazoxide treatment and, therefore, have not been included in this part of the analysis. In those neurons judged to be diazoxide insensitive, PGE₂ significantly decreased the rheobase from a control value of 1212 ± 419 to 570 ± 158 pA ($n=6$ RM ANOVA). In contrast, exposure to diazoxide had no additional effect on the rheobase in these neurons and remained at 579 ± 168 pA. There was no significant difference in the average values of the rheobase for the diazoxide-sensitive compared to the insensitive neurons under control conditions ($p=0.36$ t-test). Consistent with the observations described above, PGE₂ significantly depolarized the resting membrane potential in both subtypes (Fig. 6, bottom panel), however, only the sensitive neurons significantly hyperpolarized upon exposure to diazoxide such that the resting membrane potential returned to the resting control level. Taken together, these results indicate that a selective opener of K_{ATP} can reverse the enhanced excitability induced by PGE₂ in a subpopulation of sensory neurons.

Effects of 8-Br-cyclic GMP and glibenclamide on the sensitization produced by PGE₂

Behavioral studies of nociceptive sensitivity indicate a link between activation of the nitric oxide/cyclic GMP pathway and the antinociceptive actions of K_{ATP} (see Soares et al.

2000; Lazaro-Ibanez et al. 2001; Soares and Duarte 2001). Based on these findings, we explored the idea that 8-Br-cyclic GMP, like diazoxide, might reduce the sensitization caused by PGE₂ and that glibenclamide, an inhibitor of K_{ATP} current, could reverse this 8-Br-cyclic GMP-induced suppression. Under control conditions (Fig. 7A, top left panel), a representative neuron fired 2 APs in response to the ramp of depolarizing current. After a 5 min exposure to 1 μ M PGE₂ (top right panel), the neuron now fired 13 APs, again demonstrating that the excitability was enhanced by PGE₂. In the presence of PGE₂, exposure to 100 μ M 8-Br-cyclic GMP decreased the number of evoked APs to 5 (bottom left panel), indicating that the sensitization produced by PGE₂ was inhibited or reversed by 8-Br-cyclic GMP. In the presence of both PGE₂ and 8-Br-cyclic GMP (bottom right panel), 20 μ M glibenclamide increased the number of APs to 22. The effect of glibenclamide appeared to be reversible because the number of APs returned to levels that were observed with PGE₂ alone (13 APs). In 11 of the 21 cells, treatment with 8-Br-cyclic GMP reversed the sensitization produced by PGE₂ (based on this reversal, these neurons were identified as cGMP-sensitive neurons); these results are summarized in Fig. 7B. In these neurons, a 5 min treatment with PGE₂ significantly increased the number of APs from 2.8 ± 0.3 for the control condition to 13.5 ± 2.1 ($n=11$, RM ANOVA). In the presence of PGE₂, the number of APs was significantly decreased by 8-Br-cyclic GMP (5 min exposure) to an average value of 7.0 ± 1.4 APs ($n=11$, RM ANOVA). Subsequent exposure to glibenclamide, while in the presence of both PGE₂ and 8-Br-cyclic GMP, reversed the suppressive actions of 8-Br-cyclic GMP wherein the number of APs significantly increased to 13.0 ± 2.3 . In contrast, in 10 of the 21 cells, treatment with 8-Br-cyclic GMP did not reverse the sensitization produced by PGE₂, these neurons have been identified as cyclic GMP-insensitive neurons. In this group of neurons, the number of APs continued to increase over time and is believed to be due to the persistent actions of PGE₂. Also, in the cyclic GMP-insensitive neurons, glibenclamide had no significant effect on the number of APs and is consistent with the notion that cyclic GMP may modulate the excitability but only in those neurons expressing K_{ATP}. As above, because of the variability in the number of APs evoked from neuron to neuron, the number of APs fired under the different experimental treatments were normalized to the number of APs recorded under their respective control conditions and are shown in Fig. 7C.

In both the cyclic GMP-sensitive and -insensitive sensory neurons, the sensitization produced by PGE₂ was accompanied by a decrease in the rheobase, which is consistent with the observations reported above. As shown in Fig. 8, PGE₂ significantly decreased the rheobase from a control value of 1985 ± 467 pA to 772 ± 151 pA ($n=11$, RM ANOVA) in the cyclic GMP-sensitive neurons. Similar results were obtained in cyclic GMP-insensitive neurons wherein PGE₂ significantly reduced the rheobase to 850 ± 324 pA from a control value of 1421 ± 345 pA. Further, in the presence of PGE₂, a 5 min exposure to 100 μ M 8-Br-cyclic GMP significantly increased the rheobase from 772 pA to 1052 ± 225 pA in the cyclic GMP-sensitive neurons ($n=11$, RM ANOVA) but had no effect on the insensitive cells (850 ± 324 pA, $p=0.91$, t -test, $n=10$). Consistent with the observation that glibenclamide reversed the suppression of excitability by 8-Br-cyclic GMP, exposure to glibenclamide correspondingly decreased the rheobase in only the cyclic GMP-sensitive neurons. In the presence of PGE₂ and 8-Br-cyclic GMP, glibenclamide significantly decreased the rheobase from 1052 ± 225 pA to 740 ± 131 pA ($n=11$, RM ANOVA). Based on the actions of glibenclamide, these results indicate that cyclic GMP could suppress the enhanced excitability produced by PGE₂ through activation of K_{ATP} currents.

3. Discussion

The results reported in this study demonstrate the presence of mRNAs for the K_{ATP} channels Kir6.1 and Kir6.2 and suggest that the sulphonylurea receptor SUR1 is associated with Kir6.1/Kir6.2 in small to medium diameter sensory neurons isolated from the young adult rat. In the

CNS, the complex of Kir6.1/SUR1 was found in the glucose-sensitive neurons of the ventral-medial hypothalamus (Lee et al. 1999; Miki and Seino, 2005), whereas in the CA1 region of the hippocampus, single-cell RT-PCR studies indicated that both Kir6.1/SUR1 and Kir6.2/SUR1 were detected (SUR2 was also detected but not assigned to a Kir.6x) (Zawar et al. 1999). Detection of the SUR is significant because they serve as receptors for the hyper- and hypoglycemic agents that act as channel agonists and antagonists, respectively. Also, neither the Kir6 nor the SUR subunit forms an active/conducting channel by itself (Inagaki et al. 1995; Ammala et al. 1996). Consistent with the mRNA measurements are the electrophysiological studies wherein diazoxide, an opener of K_{ATP} channels, and FCCP, an agent that decreases intracellular ATP levels (see Gribble et al., 1997), both lead to a decrease in the number of APs evoked by a ramp of depolarizing current. The concentrations used for these agents are similar to those that have been shown to be effective in studies examining K_{ATP} channel activity in heterologous expression systems (see Edwards and Weston 1993, 1995; Inagaki et al. 1996; Gribble et al. 1997; Babenko et al. 1998). These data suggested that SUR1 combined with Kir6.1 or Kir6.2 and was modulated by a K_{ATP} channel opener. Although they did not examine effects of K_{ATP} activation on neuronal excitability, Sarantopoulos et al. (2003) recently found that in DiI positive sensory neurons isolated from the L4-5 DRGs, pinacidil but not diazoxide, which are well established agonists of K_{ATP} , enhanced the currents obtained with depolarizing voltage steps, and that these currents were reduced by glibenclamide. In contrast, our studies found that diazoxide significantly hyperpolarized the resting membrane potential, increased the rheobase of the action potential, and enhanced the amplitude of the current evoked by the voltage ramp measured at -110 mV in rat DRG neurons. Thus, our data differs from the results of Sarantopoulos et al. 2003, in this respect. The difference may result from the three distinct experimental designs. First, the size of neurons which we used is different. Sarantopoulos used neurons that had diameters ranging from 30 to 40 μ m (35.4 ± 3 μ m) whereas we used neurons in which the diameter was smaller than 35 μ m. Second, we examined a larger numbers of neurons and were able to increase our statistical sensitivity. Third, the neurons which we detected were sensitive to the capsaicin. Also, we have detected the mRNA for K_{ATP} channels. Thus, these observations demonstrate that small to medium diameter sensory neurons exhibit potassium currents that are conducted by members of the K_{ATP} family.

Based on our observations, activation of K_{ATP} may play an important role in reversing the enhanced excitability produced by pro-inflammatory agents, such as PGE_2 , in small to medium diameter capsaicin-sensitive sensory neurons. It is well established that PGE_2 augments the excitability of these nociceptive sensory neurons through an enhancement of the tetrodotoxin-resistant sodium current (TTX-R I_{Na}) (Gold et al. 1996a; England et al. 1996) and a suppression of potassium currents (Gold et al. 1996b; Nicol et al. 1997; Evans et al. 1999). It is interesting that treatment with diazoxide antagonized the PGE_2 -induced increase in the number of evoked APs in only a subpopulation of sensory neurons. Similar results were obtained for the antagonism produced by cyclic GMP. These results suggest that there may be specific subpopulations of sensory neurons that express K_{ATP} and thereby are sensitive to modulation of neuronal activity through activation of this pathway. Furthermore, activation of K_{ATP} channels potentially modulates the resting membrane potential under inflammatory conditions, which are induced by PGE_2 treatment. The sensitization produced by PGE_2 may be reversed by the capacity of K_{ATP} to hyperpolarize the resting membrane potential and/or increase the rheobase of the action potential firing; these actions would decrease the excitability. Consistent with the reduction in excitability by K_{ATP} channels, Allen and Brown (2004) reported that in magnocellular cholinergic neurons of the basal forebrain, activation of K_{ATP} produced hyperpolarization of the membrane with a concomitant reduction in excitability. Their results also suggested that K_{ATP} was tonically active at rest. Taken together, these results would suggest that K_{ATP} is active at voltages at or near the normal resting potential and may play a significant role in regulating the state of excitability in this subpopulation of sensory neurons.

The anti-nociceptive role for K_{ATP} was demonstrated in a variety of studies examining the behavioral assessment of the effects of K_{ATP} agonists and antagonists on the enhanced nociceptive response produced by inflammatory mediators (see Introduction). In addition to the behavioral studies, the capacity of K_{ATP} to directly modulate the activity of sensory neurons was indicated by the findings that activation of K_{ATP} elevated the mechanical threshold of primary afferents that triggered reflex voiding of the bladder (Yu and de Groat 1998,1999). Recently, Yu et al. (2004) demonstrated that a transurethral treatment with capsaicin increased the extent of extravasation (an indicator of neurogenic inflammation) measured in the bladder and that pretreatment with the K_{ATP} opener, ZD6169, reversed the capsaicin-induced extravasation. Treatment with glibenclamide partially reversed the decrease in extravasation. Yu et al. speculated that activation of K_{ATP} reduced the neural activity that was responsible for controlling the release of substance P and therefore reduced the extent of neurogenic inflammation. These recent findings by de Groat's laboratory regarding the capsaicin-induced sensitization of bladder afferents are consistent with our observations wherein the enhanced excitability produced by inflammatory conditions can be reduced by activation of K_{ATP} .

Previous work has demonstrated that elevations in intracellular cyclic GMP can result in the activation of K_{ATP} . In vascular smooth muscle, treatment with isosorbide dinitrate, a stimulator of soluble guanylyl cyclase, resulted in the activation of K_{ATP} and this effect was blocked by methylene blue, an inhibitor of guanylyl cyclase (Kubo et al. 1994). Similarly, treatment of mesenteric arteries with nitric oxide or lemakalim (a K_{ATP} activator) hyperpolarized the muscle membrane (Murphy and Brayden, 1995). This hyperpolarization was suppressed by either oxyhemoglobin (a nitric oxide scavenger) or by glibenclamide. Armstead (1997) reported similar findings for the actions of nitric oxide or cyclic GMP on the activation of K_{ATP} in pial artery after hypoxia. In further support for the activation of K_{ATP} by cyclic GMP and its role in reducing neuronal excitability, treatment with dibutyryl cyclic GMP could reverse, in a dose-dependent manner, the mechanical hyperalgesia that was produced by injection of PGE_2 into the hindpaw of a rat (Soares and Duarte 2001). The anti-nociception caused by cyclic GMP was antagonized by inhibitors of K_{ATP} . These different observations indicate that increased cyclic GMP can activate K_{ATP} and that this results in a reduction or suppression of excitability in a variety of tissues.

Cyclic GMP has been shown to modulate other membrane currents in sensory neurons that can affect excitability. In capsaicin-sensitive trigeminal neurons clamped at a hyperpolarized resting membrane potential of -80 mV, exposure to CPT-cyclic GMP appears to decrease the number of APs evoked by a ramp of current (Liu et al. 2004). This reduced excitability was believed to be associated with a CPT-cyclic GMP-induced suppression of the total I_{Na} and the TTX-R I_{Na} (also CPT-cyclic GMP supposedly reduced the I_A -type potassium current, which should increase excitability). In contrast to the observations of Liu et al., Renganathan et al. (2002) reported that in small diameter sensory neurons isolated from the DRG, application of 8-Br-cyclic GMP significantly increased the amplitude of the fast or TTX-sensitive sodium current but had no effect on the slow or TTX-R I_{Na} . It is unclear why two membrane permeant analogs of cyclic GMP have such different effects on these sodium currents. Perhaps, there are important differences in the modulatory effects of cyclic GMP in these two different neuronal preparations. Furthermore, based on the findings of Liu et al. (2004), the reduction in excitability produced by 8-Br-cyclic GMP (as shown in our Fig. 7) could result from the effect of cyclic GMP on TTX-R I_{Na} rather than K_{ATP} . However, TTX-R sodium channels are expressed in $\sim 80\%$ of the small diameter sensory neurons (Novakovic et al. 1998; Dib-Hajj et al. 1998). If the actions of cyclic GMP were on TTX-R I_{Na} only, then we would have expected to encounter $\sim 80\%$ of the neurons to be sensitive to the inhibitory effects of cyclic GMP whereas we found that the sensitization produced by PGE_2 was reversed by 8-Br cyclic GMP in $\sim 50\%$ of the small to medium diameter neurons (11 of 21). In addition, the notion that the effects of cyclic GMP were mediated by its actions on TTX-R I_{Na} are inconsistent with the

behavioral studies demonstrating that the anti-nociception caused by cyclic GMP was antagonized by inhibitors of K_{ATP} (Soares and Duarte 2001).

In contrast to our understanding of the signalling mechanisms involved in the initiation of PGE_2 -induced sensitization, very little is known about the transduction pathways leading to the termination or reversal of sensitization. In behavioral studies of nociception, the role of the nitric oxide/cyclic GMP pathway in modulating the hyperalgesia produced by inflammatory mediators or nerve injury is complicated by what appear to be opposing results. Some studies have found that treatment with PGE_2 heightened the sensitivity to mechanical stimuli and that this sensitization was reversed (an analgesic effect) by activation of the nitric oxide /cyclic GMP pathway (Ferreira and Nakamura 1979;Duarte et al., 1990;Duarte et al., 1992). At the level of the spinal cord, the production of nitric oxide and cyclic GMP are required for the sustained thermal, but not mechanical, hyperalgesia (Meller and Gebhart, 1993;Meller et al., 1994). Thus, it would seem that cyclic GMP in the periphery was antinociceptive whereas cyclic GMP was pronociceptive in the spinal cord. In all likelihood, our recordings were obtained from sensory neurons innervating cutaneous targets wherein we observed that cyclic GMP reduced the augmented excitability and would be consistent with an antinociceptive role. This exact role of cyclic GMP is complicated further by recent findings. Intrathecal delivery of SIN-1, a nitric oxide donor, was capable of reducing the mechanical hyperalgesia resulting from injury to the sciatic nerve (Sousa et al 2001). In addition, intrathecal 8-Br-cGMP suppressed the flinching behavior produced by the injection of formalin into the hind paw (Tegeda et al. 2002). Interestingly, these antinociceptive effects were produced by low dosages of these agents, however, higher dosages of SIN-1 or 8-Br-cyclic GMP had pronociceptive effects in these animal models (Sousa et al 2001;Tegeda et al. 2002). Furthermore, the exact nature of the analgesic properties of cyclic GMP in the periphery are now unclear. Intradermal injection of PGE_2 produced a hypernociception to mechanical stimuli within ~15 min whereas subcutaneous injection resulted in mechanical hypernociception that peaked in intensity after 2-3 hr (Khasar et al. 1993;Aley et al. 1998;Vivancos et al., 2003). Treatment with SIN-1 suppressed the hypernociception resulting from the subcutaneous injection (Vivancos et al., 2003), however, the nitric oxide donor enhanced the hypernociception produced by the intradermal injection (Aley et al. 1998;Vivancos et al., 2003). These results suggest that there may be two populations of C-fibers innervating these tissues whose sensitivity to nitric oxide may result in very different nociceptive outcomes after exposure to inflammatory mediators. In these animal models, interpretation of the results regarding both the cell types undergoing modulation as well as the dissection of specific cellular signalling pathways thought to mediate these actions are complicated by the complexity of the many tissues involved in the nociceptive response.

In conclusion, small to medium diameter sensory neurons express the mRNA for Kir6.1 and Kir6.2; these channels are likely associated with SUR1. Although PGE_2 enhanced the excitability of these small to medium diameter sensory neurons, activation of K_{ATP} suppressed this sensitization in only a subpopulation of neurons. In terms of a physiological role for K_{ATP} in nociceptive sensory neurons, one can speculate that inflammation or nerve injury gives rise to conditions that are associated with altered neuronal metabolism, stress, or increased activity. Such conditions could produce a fall in intracellular ATP levels and/or stimulate signalling pathways capable of increasing intracellular cyclic GMP that then lead to activation of K_{ATP} . This current causes hyperpolarization of the neuron or an alteration in membrane resistance that consequently reduces the enhanced excitability. Therefore, activation of K_{ATP} may provide a mechanism to reverse or antagonize the sensitization resulting from injury or decrease the potential of the neuronal hyperexcitability induced by these conditions.

4. Experimental procedures

Isolation and maintenance of adult rat sensory neurons

Sensory neurons were isolated from young adult male rats using procedures developed by Lindsay (1998) with slight modifications. Briefly, young adult male Sprague-Dawley rats (100-150 g) were killed by placing them in a chamber filled with CO₂. The isolated spinal column was hemisected, the spinal cord was removed, and the dorsal root ganglia (DRG) were collected in a culture dish filled with sterilized Puck's solution (calcium-free, magnesium-free Hank's balanced salt solution). The ganglia were transferred to a conical tube with F-12 media containing papain (20 U/ml) and incubated for 15 min at 37° C, followed by incubation in 1 mg/ml collagenase IA and 2.5 mg/ml dispase for 10 min. The tube was centrifuged for 30 s before the enzyme-containing supernatant was removed. The pellet was resuspended in F-12 media supplemented with 250 ng/ml nerve growth factor (Harlan Bioproducts for Science, Indianapolis, IN) and mechanically dissociated with fire-polished pipettes until all obvious chunks of tissue were gone. Isolated cells (~150,000 cells/ml) were plated on plastic cover slips coated with poly-D-lysine and laminin. The cells were maintained in F-12 media at 37° C and 3% CO₂ and used within 1-2 days for experiments. All procedures were approved by the Animal Use and Care Committee of Indiana University School of Medicine.

Electrophysiology

Recordings were made using the perforated patch-clamp technique (Horn and Marty 1988;Rae et al. 1991;Lopshire and Nicol 1997). Briefly, a cover slip with the sensory neurons was placed in a recording chamber where neurons were bathed in normal Ringers of the following composition (in mM): 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES and 10 glucose, pH at 7.4 with NaOH. The tip of the electrode was filled with an internal solution composed of the following (in mM): 140 KCl, 1 MgCl₂, 10 HEPES, pH 7.4 adjusted with KOH. The electrode was then backfilled with the internal solution containing 120 µg/ml amphotericin B, and used according to procedures outlined in Horn and Marty (1988) and Rae et al. (1991). These electrodes had resistances between 2 and 4 MΩ in normal Ringers. After attaining the cell-attached configuration, the partitioning of amphotericin B into the membrane was assessed by applying +10 mV steps (duration 80 ms) from a holding potential of -60 mV at 2 min intervals until a steady-state access resistance was obtained (typically 15 min).

In current clamp experiments, changes in membrane voltage were recorded using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA); neurons were held at their resting potentials. To assess the excitability, a ramp of depolarizing current (1 s in duration) was applied to the neuron wherein the amplitude of the ramp was adjusted to produce three to six action potentials (APs) under control conditions. The amplitude then remained constant throughout the recording period for each individual neuron. These recordings were sampled at 1-2 kHz using pClamp 8.0 (Axon Instruments). At the end of each recording, 100 nM capsaicin was focally applied to the neuron for ~2 seconds. If the neuron depolarized by at least 5 mV and/or generated APs, then it was judged to be capsaicin sensitive. This neurotoxin was used to distinguish capsaicin-sensitive sensory neurons as these neurons are believed to transmit nociceptive information (Holzer, 1991). The electrophysiological results reported below were obtained from only capsaicin-sensitive neurons. All experiments were performed at room temperature (~23° C).

In voltage clamp experiments, to isolate potassium currents, all recordings were obtained in a Ringers solution wherein N-methylglucamine-Cl (NMG) replaced NaCl on an equimolar basis. Cells were voltage-clamped and held near their normal resting membrane potential. To assess K_{ATP}, a voltage protocol was used wherein the membrane potential was held at -60 mV, a 450 ms ramp that began at -120 mV and increased to 20 mV.

RT-PCR

For those studies determining mRNA expression in the intact ganglia, the DRGs were collected from the entire spinal column. The ganglia were then disrupted with a glass homogenizer. The total RNA was isolated from the DRGs using the RNeasy mini kit and treated with RQ1 RNase-free DNase I, according to the instructions provided by the manufacturer (Qiagen, Chatsworth, CA). After isolation, 1 µg of the total RNA was reverse transcribed using an iScript™ cDNA synthesis kit (Bio-Rad, Hercules, CA), according to the manufacturer's instructions. For the RT-PCR experiments with individual neurons, a single neuron was collected with specially prepared micropipettes (20-25 µm tip diameter). A plastic cover slip on which the neurons were grown was transferred to the recording chamber and superfused with normal Ringers prepared with DEPC water for 10 min. Under visual control, a micropipette filled with DEPC-treated water was positioned above a small to medium diameter sensory neuron (<35 µm diameter). Negative pressure was applied to aspirate the cell into the tip of the pipette. A single cell was collected for each RT-PCR experiment. The solution with the collected cells was transferred to a 0.2 ml PCR tube. Using the procedure developed by Song et al. (1998) with slight modification, the total RNA from one neuron was treated with a DNase 1 amplification grade kit (Invitrogen Corp), according to the manufacturer's instructions. To increase the efficiency of reverse transcription, a "high throughput" protocol was used, wherein random hexamers were included so that the reaction buffer contained 1 µl of oligo(dt) 12-18, 1 µl dNTP, 1 µl random hexamers, 2 µl 10X buffer, 2 µl dTT, 1 µl RNaseout, and 1 µl Super Script RT. This mixture was incubated at 25° C for 10 min, then at 42° C for 50 min, and followed by 10 min at 70° C. At the end of each reaction, a 5 µl aliquot of the cDNA product was either used in the PCR step or stored at -20° C. These reagents were obtained from Invitrogen Corp. (Carlsbad, CA). The primers were as follows: for Kir6.1 (accession number [D 42145](#)): forward primer (bases 1292-1311), GAGTGAAGTGTGCGACCAGA; reverse primer (bases 1539-1520), CGATCACCAGAACTCAGCAA; for Kir6.2 (accession number [X 97041](#)): forward primer (bases 787-804), TCCAACAGCCCGCTCTAC, reverse primer (bases 954-937), GATGGGGACAAAACGCTG; for SUR1 (accession number [L 40624](#)): forward primer (bases 4774-4792), TGAAGCAACTGCCTCCATC, reverse primer (bases 4955-4937), GAAGCTTTCCGGCTTGTC; and for SUR2 (accession number [D 83598](#)): forward primer (bases 4853-4872), ACCTGCTCCAGCACAAGAAT, reverse primer (bases 4997-4976), TCTCTTCATCACAATGACCAGG. The PCR protocol was 94° C for 1 min, 51° C for 1 min, 72° C for 2 min, for a total of 45 cycles. The PCR products were separated on 1.5% agarose gels. The amplified PCR products were sequenced in both directions using the ABI Prism 3100 genetic analyzer capillary DNA sequencer.

Data analysis

All values represent the mean ± standard error of the mean (SEM). To determine the rheobase, the dV/dt transform of the voltage recording was performed. The value of the voltage that exceeded the baseline value of dV/dt by >20-fold was taken as the firing threshold. The corresponding current value on the ramp was then measured as the rheobase. The baseline dV/dt was determined by averaging the points between the onset of the ramp and the following 100 ms. Statistical differences between the control recordings and those obtained after treatment conditions were determined by using a paired t-test, a one-way ANOVA, or a repeated measures ANOVA (RM ANOVA) wherever appropriate. When a significant difference was obtained with an ANOVA, a post-hoc analysis was performed using a Tukey or Rank test. Values of p<0.05 were judged to be significant.

Chemicals

PGE₂ was obtained from Cayman Chemical Co. (Ann Arbor, MI). All other chemicals were obtained from Sigma Chemical Corp. (St Louis, MO). Stock solutions of diazoxide,

glibenclamide, PGE₂ and capsaicin were made by dissolving these agents in 1-methyl-2-pyrrolidinone and then diluting further in the appropriate Ringers solution on a daily basis. The final concentration of vehicle in the test solution was <0.1%, which had no effect on the voltages obtained in control experiments (see Nicol et al. 1997; Zhang et al. 2002).

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Abbreviations

APs, action potentials; ANOVA, analysis of variance; DRG, dorsal root ganglia; FCCP, mesoxalonnitrile 4-trifluoromethoxyphenylhydrazide; K_{ATP}, ATP-sensitive potassium current; L4-6, lumbar 4-6 DRGs; PGE₂, prostaglandin E₂; RM ANOVA, repeated measures analysis of variance; SEM, standard error of the mean; SUR, sulphonylurea regulatory subunit; TTX-R I_{Na}, tetrodotoxin-resistant sodium current.

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Figure 1.

Sensory neurons express the mRNA for the SUR subtypes SUR1 and SUR2. Panel A illustrates that both SUR1 (S1) and SUR2 (S2) were detected in a sample from an intact ganglia preparation (labelled total) whereas no PCR products were detected in the blanks (B). Panel B shows that the mRNA for SUR1 was detected in two different samples obtained from two groups of five small to medium diameter sensory neurons (SUR1 lanes 1 and 3). SUR1 was also detected in a sample (lane 2) obtained from a different intact ganglia preparation than shown in panel A. The primer pair for SUR2 did not detect SUR2 in a sample from five small to medium diameter sensory neurons (SUR2 lane 1, same cDNA as used for SUR1 lane 3). However, SUR2 was detected (SUR2 lane 2) in the sample from the same intact ganglia that exhibited SUR1 (SUR1 lane 2). Samples that did not contain any cDNA are shown as the blanks (lanes labelled B). Panel C demonstrates that in the presence of reverse transcriptase (+RT), PCR products for SUR1 were detected in total cDNA from whole ganglia (lanes 1 and 2) and from two (lanes 4 and 6) single cell RT-PCR samples but not in three others (lanes 3, 5, and 7). In the absence of reverse transcriptase (-RT), no products were detected for either total cDNA (lanes 1 and 2) or for single cells (lanes 3-5). No products were detected in the

blank samples (lanes labelled B). For all panels, the base pair ladder (labelled bp) is shown on the right.

Figure 2.

Sensory neurons express the mRNA for the K_{ATP} channel subtypes Kir6.1 and Kir6.2. The left panel shows the mRNA for Kir6.1 in individual single cells (labeled S1, S2, S3, S4 and S5) and an intact ganglia preparation (labeled total). The middle panel shows the mRNA for Kir6.2 in individual single cells (labeled S1, S2, S3, S4 and S5) and in the same intact ganglia preparation as shown in the left panel (labeled total). For all panels, the base pair ladder (labeled bp) is shown on the right. No PCR products were detected in the no template control (lanes labeled B).

Figure 3.

Diazoxide, a K_{ATP} channel opener, reduces the excitability of small to medium diameter capsaicin-sensitive sensory neurons. Panel A illustrates a current clamp recording from a representative neuron wherein the ramp of depolarizing current elicits 7 APs whereas after a 5 min exposure to 300 μ M diazoxide the number is reduced to 3. Note that the resting membrane potential hyperpolarizes by 5 mV after diazoxide. The dotted line represents the level of 0 mV. The panels shown in B summarize the results obtained for those neurons that were either sensitive (n=8) or insensitive (n=9) to diazoxide. The top panel represents the effects of a 5 min exposure to 300 μ M diazoxide on the number of APs evoked by the ramp. After treatment 3 of 8, diazoxide-sensitive neurons did not fire any APs (see Results). The middle panel

demonstrates that diazoxide hyperpolarizes the resting membrane potential in those sensitive neurons. The bottom panel shows the effects of diazoxide on the normalized rheobase where the rheobase after diazoxide is divided by their respective control values. The asterisks indicate a significant difference from the control values ($p < 0.05$).

Figure 4.

Diazoxide increases the inward K_{ATP} current in small to medium diameter capsaicin-sensitive sensory neurons. Top left panel in A shows an example of the inward current evoked by the voltage ramp (left bottom panel in A) from a representative neuron for the control and after a 5 min exposure to 300 μ M diazoxide. This enhancement of the current by diazoxide is shown at greater resolution in the right panel of A. Panel B summarizes the amplitude of the currents obtained for those neurons that were either sensitive ($n=9$) or insensitive ($n=6$) to diazoxide measured at -110 mV. Panel C illustrates the results obtained with diazoxide for these same neurons when the current values have been normalized to their respective control values measured at -110 mV. The asterisks indicate a significant difference from the control values ($p<0.05$).

Figure 5.

Diazoxide reverses the sensitization produced by PGE₂. The top left panel of A shows a current-clamp recording from a representative small to medium diameter capsaicin-sensitive sensory neuron. A ramp of depolarizing current (1 s duration, 5000 pA final amplitude) was used to evoke APs under control conditions. The top right panel demonstrates the increase in the number of evoked APs after a 5 min treatment with 1 μ M PGE₂. The bottom left panel shows the reduction in the number of APs after a 5 min exposure to 300 μ M diazoxide (Diaz) in the presence of PGE₂. The bottom right panel demonstrates that a 5 min washout with normal Ringers can reverse the reduction in APs produced by diazoxide. The numbers to the left of each trace represent the resting membrane potential; the dotted lines indicate the 0 mV level;

the calibration scales in the middle is for the top and bottom right traces whereas the scale to the left is for the bottom left panel only. The effects of these different treatments on the number of evoked APs are summarized in panel B. Panel C illustrates the effects of these treatment conditions after the number of APs have been normalized to that obtained for each neuron under their respective control conditions. The asterisks represent a significant difference between the values obtained under control conditions and after a particular treatment ($p < 0.05$, RM ANOVA). The # represents a significant difference between the PGE₂ and PGE₂ plus diazoxide treatments ($p < 0.05$ RM ANOVA).

Figure 6.

Diazoxide reverses the PGE₂-induced increase in excitability. The top panel shows that diazoxide decreases the rheobase after diazoxide when normalized to their respective control values. Sensory neurons were sequentially exposed to 1 μ M PGE₂ for 5 min, then 1 μ M PGE₂ plus 300 μ M diazoxide. The bottom panel shows that diazoxide reverses the PGE₂-induced depolarization of the resting membrane potential (RMP). The asterisks represent a significant difference between the values obtained under control conditions and after a particular treatment ($p < 0.05$, RM ANOVA). The # represents a significant difference between the PGE₂ and PGE₂ plus diazoxide treatments ($p < 0.05$ RM ANOVA).

Figure 7.

In a subpopulation of sensory neurons, 8-Br-cGMP reverses the sensitization produced by PGE₂; subsequent treatment with glibenclamide can reverse the suppressive effects of 8-Br-cGMP. Panel A illustrates, in a representative neuron, that under control conditions the depolarizing ramp evoked 2 APs (top left); a 5 min exposure to 1 μ M PGE₂ produced sensitization as indicated by the increased number of APs (top right). Treatment with 100 μ M 8-Br-cGMP (5 min) reduced the increase in the number of APs produced by PGE₂ (bottom left), but only in a subpopulation of sensory neurons. Subsequent treatment with 20 μ M glibenclamide for 5 min reverses the suppressive effects of 8-Br-cGMP (bottom right panel). APs were evoked by a ramp of current that was 1 sec in duration with a final amplitude of 5000

pA. Each dotted line indicates 0 mV. The values at the beginning of each trace represent the resting membrane potential. Panel B summarizes the effects of sequential treatment of sensory neurons with PGE₂, PGE₂ + 8-Br-cGMP, and finally PGE₂ + 8-Br-cGMP + glibenclamide on the number of APs evoked by the ramp. Panel C represents the effects for the different treatment conditions after they have been normalized to their respective control responses. Asterisks represent a significant difference between values obtained under control and the different treatment conditions ($p < 0.05$, RM ANOVA). The # represents a significant difference between the values obtained for the PGE₂ + 8-Br-cGMP condition vs. PGE₂ alone or the PGE₂ + 8-Br-cGMP + glibenclamide ($p < 0.05$, RM ANOVA).

Figure 8.

8-Br-cGMP reverses the PGE₂-induced decrease in rheobase of neurons. These results were obtained from those same neurons represented in Fig. 7. Asterisks represent a significant difference between values obtained under control and the different treatment conditions ($p < 0.05$, RM ANOVA). The # represents a significant difference between the values obtained for the PGE₂ + 8-Br-cGMP condition vs. PGE₂ alone or the PGE₂ + 8-Br-cGMP + glibenclamide ($p < 0.05$, RM ANOVA).