Evidence that Tolerance and Dependence of Guinea Pig Myenteric Neurons to Opioids Is a Function of Altered Electrogenic Sodium-Potassium Pumping

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Accepted for publication October 21, 1996

ABSTRACT

Ouabain acutely depolarizes most types of cells through inhibition of electrogenic Na⁺,K⁺ pumping and is a useful tool with which to study conditions that affect electrogenic pumping. Intracellular recording techniques were used with neurons of the guinea pig myenteric plexus/longitudinal muscle preparation exposed to ouabain. Of 35 S neurons exposed to ouabain (1 μM), 15 were hyperpolarized by 10 ± 2 mV, 11 were depolarized by 8 ± 2 mV and the remaining neurons had no change in membrane potential. The nonselective potassium channel antagonist tetrodylammonium chloride (TEA; 0.5 mM) alone evoked modest (~5 mV) and inconsistent changes in the resting membrane potential of S neurons. However, in the presence of TEA, the hyperpolarizing response to 1 μM ouabain was eliminated, and the proportion of cells depolarized by ouabain increased from 31% to 83%. Gilbenclamide (10 μM) and 100 nM iberiotoxin did not change the pattern of membrane potential changes induced by 1 μM ouabain. Calcium-free buffer eliminated the hyperpolarization and potentiated the depolarization induced by 1 μM ouabain. Ouabain (5 μM), in either the presence or absence of TEA, induced depolarization in all neurons tested (mean, 15–16 mV), indicating a predominant effect of inhibition of electrogenic pumping. These data suggest that ouabain may directly or indirectly activate myenteric S neuron calcium-sensitive potassium channels as well as inhibit the Na⁺,K⁺ pump and that TEA will antagonize the former effect. Chronic exposure (morphine pellets) of guinea pigs to morphine resulted in a partial depolarized state of myenteric neurons, as previously reported. Ouabain (5 μM), either with or without TEA, depolarized neurons from chronically morphine-treated guinea pigs very little (5–6 mV) in comparison with naive neurons (15–16 mV). This supports the conclusion that the depolarized state of morphine-tolerant neurons is associated with a reduction in electrogenic Na⁺,K⁺ pumping.

Past work in our laboratory (Abel et al., 1981; Fleming and Westfall, 1975; Gerthoffer et al., 1979) and others (Albuquerque et al., 1971; Sellin and Thesleff, 1980; Rogers et al., 1993) has established that the mechanism by which nonspecific adaptive supersensitivity occurs in several smooth muscles and skeletal muscles is partial membrane depolarization. Furthermore, in smooth muscle, that depolarization has been proved to be the consequence of altered Na⁺,K⁺ pump function, including a marked decline in electrogenic pumping (Abel et al., 1981; Gerthoffer et al., 1979; Hershman et al., 1993, 1995; Rogers et al., 1993). The cardiac glycylsides, such as ouabain, by means of their inhibition of the pump, have been a critical tool in much of that work.

Recently, our laboratory has established that nonspecific adaptive supersensitivity to neuronal excitatory agents and subresponsivity to inhibitory agents, including opioids, are the bases for the tolerance to, and dependence on, opioids that develop in the myenteric plexus/longitudinal muscle preparation of the guinea pig (Johnson et al., 1978; Taylor et al., 1988). Subsequently, depolarization of myenteric S neurons was identified as the mechanism for those sensitivity changes (Leedham et al., 1992). Investigation of the important issue of the role of electrogenic Na⁺,K⁺ pumping in this effect has been complicated by the fact that ouabain causes a hyperpolarization, rather than a depolarization, in many S neurons.

The resting membrane potential is the sum of two components: (1) the diffusion potential that is driven by the transmembrane potassium gradient and is maintained, over the long term, by Na⁺,K⁺-ATPase (EC 3.6.1.37) and (2) the electrogenic pump potential. Because of the unequal ionic pumping, an outward current, the electrogenic pump current, is generated by the Na⁺,K⁺ pump, contributing to the potential difference across the membrane (Fleming, 1980; Thomas, 1972). It has been estimated that electrogenic pumping con-

ABBREVIATIONS: TEA, tetrodylammonium chloride monohydrate; PSS, physiological salt solution; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N',N'-tetraacetic acid.
Cardiac glycosides, such as ouabain, specifically bind to the Na\(^+\),K\(^+\)-ATPase, inhibiting both the enzyme activity and pump function (Allgayer and et al., 1988; Benders et al., 1992). In a variety of cells, including neurons, muscles and glands, ouabain induces a rapid depolarization by inhibiting the electrogenic pumping of the Na\(^+\),K\(^+\)-ATPase (Brodie and Sampson, 1985; Bryant et al., 1988; Fleming, 1980; Miller et al., 1978; Xu and Adams, 1992). Although we observed ouabain-induced depolarizations in some guinea pig myenteric-type S neurons, approximately half of the neurons were hyperpolarized by ouabain. Similar results have been reported in rabbit nodose ganglion neurons (Higashi et al., 1987). These authors found that ouabain induced extracellular calcium influx or intracellular calcium release from nodose ganglion C neurons. The increase in intracellular free calcium activated a calcium-dependent potassium conductance that resulted in hyperpolarization. We hypothesize that in guinea pig myenteric S neurons, in addition to inhibition of the Na\(^+\),K\(^+\) pump, which should lead to depolarization, ouabain might also, directly or indirectly, activate a potassium channel. This increase in potassium conductance might contribute to the hyperpolarization of the neuron induced by ouabain.

The present work was undertaken with two objectives: (1) to determine the cause of the hyperpolarizing effect of ouabain and a method to eliminate or minimize it and (2) to use those results to design experiments to investigate the role of electrogenic pumping in the depolarization associated with opioid tolerance. In choosing the range of concentrations of ouabain (1–5 \(\mu M\)), the goal was to achieve near-maximal inhibition of the pump. Experience in other tissues of the guinea pig indicated that the \(K_D\) for ouabain binding to the pump was 0.1–0.2 \(\mu M\) (Wong et al., 1981), and electrogenic pump inhibition was complete at 10 \(\mu M\) (Gertshoffer et al., 1979; Urquilla et al., 1978). Preliminary results have been presented in abstracts (Kong et al., 1993, 1996).

Methods

Myenteric neuron electrophysiology. Adult male albino guinea pigs (Hilltop Lab Animals, Inc., Scottsdale, PA; Camm Research Lab Animals, Wayne, NJ) weighing 350–600 g were used. Guinea pigs were killed by exsanguination after stunning. The technique for tissue preparation was previously described by Leedham et al. (1992) and similar to that of Cherubini and North (1984) and Johnson et al. (1987). The ileocecal junction was identified and \(-10\) cm of terminal ileum was discarded. The adjacent 10 cm of ileum was removed, and the lumen was flushed with physiological saline. Segments of ileum \(-1\) cm long were cut lengthwise and pinned in a tissue chamber designed for intracellular recording. After removal of the overlying mucosa and circular muscle layers, the myenteric ganglia were visualized using an Olympus inverted microscope equipped with Nomarski optics. Tissues were continuously superfused with PSS maintained at 37 ± 1°C, bubbled with 95% O\(_2\)/5% CO\(_2\), at a constant flow rate of 2 ml/min with a Gilson Minipulse II peristaltic pump (Rainin Instrument, Woburn, MA). The composition of the PSS was 117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\), 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 25 mM NaHCO\(_3\) and 11.5 mM glucose. In addition, nicardipine (3 \(\mu M\)) was added to the PSS to reduce or prevent muscle contractions. In the calcium-free medium, calcium ion was substituted by sodium ion, and 2 mM EGTA was added to the medium. Tissue exposure to ouabain was achieved by changing the perfusate from one without the drug to one containing the drug. The time required to achieve a complete exchange of solution in the tissue chamber, due to the dead space in the superfusion system, was 1–2 min. Neurons of the myenteric ganglia were impaled using glass fiber-filled microelectrodes (Frederick Haer, Brunswick, ME) filled with 2.5 M KCl and having resistances of 50–100 MΩ. AH cells were classified on the basis of the appearance of a long-lasting (2–30 sec) afterhyperpolarization following an action potential evoked in the soma by intracellular depolarizing current pulse injection (Hirst et al., 1974). Neurons were identified as S cells if repetitive intracellular depolarizing current injection could evoke repetitive somatic action potentials without afterhyperpolarizations and/or by the presence of fast excitatory postsynaptic potentials elicited by transmural stimulation. Although some S neurons do display an afterhyperpolarization (Cherubini et al., 1988), in our experiments this is not as great or as long lasting as in AH cells, nor does it prevent repetitive generation of action potentials. Only cells identified as S neurons were exposed to ouabain. The methods used for intracellular recording and cell identification are similar to those previously reported by Hirst et al. (1974), Cherubini and North (1984), Johnson et al. (1987) and Leedham et al. (1992). Signals from these neurons were amplified (Axoclamp 2A, Axon Instruments, Burlingame, CA) and displayed on a Tektronix Model 5111 oscilloscope. A continuous record of the amplifier output was obtained via a digital computer/AV interface board and Axotape data acquisition software (Axon Instruments, Burlingame, CA). Measurements of resting membrane potential (resting \(E_m\)) and changes in \(E_m\) induced by superfusion with drugs could be obtained from computer recordings. Membrane input resistance was calculated from the amplitude of the voltage response to a constant hyperpolarizing current pulse injection (0.1–0.3 nA, 250 msec, 0.3 Hz). Currents were used that evoked voltage responses of \(>20\) mV in amplitude under control conditions. Cells were included in the analysis if the following criteria were met: (1) resting membrane potential of the cell remained steady for \(>15\) min, (2) the stable resting potential difference was \(>40\) mV and (3) the peak amplitude of the action potential elicited by intracellular current injection surpassed 0 mV. Statistical evaluations were made using Student’s \(t\) tests for unpaired samples. Paired Student’s \(t\) tests were also used where appropriate. A value of \(P \leq .05\) was accepted as significant.

Morphine pellet implantation. Morphine pellets were implanted subcutaneously in guinea pigs anesthetized with 0.1 ml/100 g b.wt. of Innovar (0.05 mg/ml fentanyl citrate/2.5 mg/ml droperidol) administered subcutaneously. Each morphine pellet contained 75 mg of morphine. Under anesthesia, either one or two pellets were implanted in each flank, depending on animal weight, as previously described (Goldstein and Schulz, 1973; Johnson et al., 1978). With this method of implantation, there was very low morbidity and mortality (<1%). Animals were allowed to recover from the anesthesia and given free access to food and water until the time of experiment, 7 days later. Extensive experience has established that two pellets in the smaller guinea pigs and four in the larger guinea pigs produce equivalent tolerance in the myenteric plexus/longitudinal muscle preparation. The method produces peak tolerance over a period of 4–7 days (Leedham et al., 1989).

Drugs. Glibenclamide and ibetoxitoxin were purchased from Research Biochemicals (Natick, MA). Tetraethylammonium chloride monohydrate was obtained from Aldrich Chemical (Milwaukee, WI). (Ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) was from Fisher Scientific (Fair Lawn, NJ). Ouabain octahydrate and other reagents were obtained from Sigma Chemical (St. Louis, MO). Morphine sulfate and the morphine and placebo pellets were obtained from Mr. K. H. Davis (Research Triangle Institute through the National Institute of Drug Abuse).
Results

In a concentration of 1 μM, ouabain hyperpolarized 43% of the guinea pig myenteric S neurons tested, depolarized 31% and produced no change in 26% (table 1). Figure 1 shows a typical hyperpolarization from one neuron that, in this subpopulation of S neurons, averaged 10 ± 2 mV. Membrane input resistance of neurons was monitored by measuring the voltage response to regularly repeated injections of a constant hyperpolarizing current pulse injection. In several neurons hyperpolarized by ouabain, the membrane input resistance was decreased during ouabain perfusion and gradually recovered after washing of ouabain from the preparation with normal Krebs’ solution (fig. 1). This alteration in input resistance was repeatable on the same neuron. However, no statistically significant difference was found among all sets of input resistance measurement data before and during superfusion of ouabain (1.0 μM) (table 2).

TEA (0.5 mM), a nonselective potassium channel blocker, eliminated the hyperpolarizing effect of ouabain (1 μM) such that it induced only depolarization (10 of 12 cells, 83%) or no effect (2 cells, table 1). TEA alone had minimal effects on membrane potential or input resistance. The mean increase in resistance induced by ouabain was greater in the presence of TEA (table 2), but variability was considerable, and the mean value was not significantly different from zero.

Other more-selective potassium channel blockers were tested to further define the nature of the potassium channel involved in the ouabain-induced hyperpolarization of S neurons. Glibenclamide, a sulfonylurea, is an inhibitor of ATP sensitive potassium channels (Gasser and Vaughan Jones, 1990; Yanagisawa et al., 1990). Preperfusion of the preparation with 10 μM glibenclamide for 20 min induced no obvious changes in response of S neurons to ouabain (table 1). Iberiotoxin, a toxin isolated from scorpion venom, is a selective inhibitor of high conductance calcium-activated potassium channels with a high affinity constant (Maxi-K). The IC50 value for single high conductance KCa channels on bovine aortic smooth muscle was 250 pM (Galvez et al., 1990). After preperfusion of myenteric plexus preparation with 20 nM iberiotoxin for 5–10 min, the neuronal action potential generated by depolarizing current injection was obviously broadened with a slowed repolarization phase (data not shown). This effect implies that iberiotoxin-sensitive potassium channels had been blocked by the iberiotoxin treatment. Superfusion with an even higher concentration of iberiotoxin (100 nM) for 1 hr did not reduce the frequency or magnitude of hyperpolarizing responses to ouabain (table 1). In contrast, perfusion with calcium-free medium had a dramatic effect on responses of type S neurons to ouabain. After a 20-min perfusion with calcium-free Krebs’ solution, 1 μM ouabain depolarized all seven S neurons tested by an average of 28 ± 4 mV, an amount significantly greater than the depolarization induced by ouabain in normal calcium Krebs’ solution (table 1).

A larger concentration of ouabain (5 μM) depolarized all 11 control neurons tested by a mean of nearly 16 mV (table 3, control data). In the presence of TEA, similar results were obtained with all 27 neurons depolarized by a mean of >15 mV. This concentration of ouabain induced a mean increase in input resistance of 11.3 ± 7.9 MΩ (not significantly different from zero) in the absence and 21.1 ± 6.1 (P < .05) in the presence of TEA (table 2). Although the mean depolarization induced by 5 μM ouabain was similar in the presence and absence of TEA (table 2), the relative changes in input resistance are consistent with ouabain opening some K+ channels (which decreases resistance) in the absence of TEA.

Experiments were carried out to determine whether the depolarized state of myenteric S neurons induced by implantation of morphine pellets in guinea pigs was related to a loss of electrogenic Na+,K+ pumping. The data above suggest that in these neurons, 5 μM ouabain is more selective for pump inhibition than is 1 μM and that TEA is an effective antagonist of any complicating effect of ouabain on K+ channels. A total of 38 S neurons from control animals and 13 from guinea pigs chronically exposed to morphine were studied (table 3). As previous studies have established (e.g., Leedham et al., 1992), the S neurons from the treated animals were significantly depolarized relative to control cells (table 3). In both the absence and presence of TEA, the neurons from morphine-pretreated guinea pigs were depolarized significantly less by ouabain than were controls (table 3). The differences in the depolarization induced by ouabain in the absence of TEA (15.9 – 7.4 = 8.5 mV) and the presence of TEA (15.2 – 4.5 = 10.7 mV) are very similar to the difference in resting potential between control neurons and those from guinea pigs pretreated with morphine (54.0 – 45.3 = 8.7 mV). In other words, the mean membrane potential of the 38 control neurons in the presence of ouabain (–38.6 mV) was virtually identical to the mean membrane potential of the 13 neurons from morphine-pretreated guinea pigs in the presence of ouabain (–39.3 mV). These data are consistent with a conclusion that the depolarized state of neurons from the morphine-pretreated guinea pigs is due to reduced electrogenic Na+,K+ pumping, not to altered diffusion potential.

Discussion

The primary purpose of this study was to determine whether changes in electrogenic Na+,K+ pumping contribute to the partial depolarization induced in myenteric S neurons by chronic treatment of guinea pigs with morphine. To do so first required that a complicating factor in the action of cardiac glycosides be eliminated.

Ouabain (1 μM) induced depolarization in some myenteric S neurons and hyperpolarization in others. Depolarizations

| TABLE 1 |
| Effects of ouabain (1 μM) on the resting membrane potential of s neurons under various experimental conditions |

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hyperpolarization</th>
<th>Depolarization</th>
<th>No change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain Alone</td>
<td>10 ± 2 (15)</td>
<td>8 ± 2 (11)</td>
<td>7 ± 2 (10a)</td>
</tr>
<tr>
<td>+TEA (0.5 mM)</td>
<td>8 ± 2 (6)</td>
<td>7 ± 0.4 (2)</td>
<td>3 ± 5 (4)</td>
</tr>
<tr>
<td>+Glibenclamide (10 μM)</td>
<td>9 ± 1 (3)</td>
<td>13 ± 5 (4)</td>
<td>28 ± 4 (7a)</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M. changes in mV from (n) of cells observed.

a Indicates significantly different distribution from ouabain alone by χ² analysis, P < .05.
induced by ouabain have been reported in many neurons and other cell types over several decades and have been recognized to be the result of the inhibition of electrogenic Na⁺,K⁺ pumping (e.g., Fleming, 1980; Sontheimer et al., 1994; Thomas, 1972). In contrast, ouabain-induced hyperpolarization has previously been observed in only two groups of cells: nodose ganglion neurons (Higashi et al., 1987) and colonic myocytes (Burke and Sanders, 1990). The results of Higashi et al. (1987) and Burke and Sanders (1990) and those presented here suggest that these hyperpolarizations result from the activation of potassium channels. By opening potassium channels, ouabain would change the resting membrane potential of the neurons toward the potassium equilibrium potential (i.e., induce a hyperpolarizing effect). This hypothesis is supported by the fact that TEA (a widely recognized inhibitor of many types of K⁺ channel; Watson and Girdlestone, 1996) prevents the hyperpolarizations induced by ouabain. If this hypothesis is correct, ouabain should decrease membrane input resistance by increasing potassium conductance. Although such reductions in input resistance were clearly demonstrable in some cells, no significant decrease was found when data were compared among all cells in which ouabain produced hyperpolarizations. The depolarizations induced by a higher concentration of ouabain (5 μM) were accompanied by a nonsignificant trend toward increased input resistance. However, in the presence of TEA, this increase in resistance induced by the larger concentration of ouabain was greater and significant. It is likely, therefore, that the tendency of ouabain to increase resistance was masking a small decrease in resistance due to opening of potassium channels in some of the cells.

Hyperpolarization induced by ouabain in rabbit nodose ganglion type C neurons has been reported by Higashi et al. (1987), who concluded that ouabain indirectly activated a calcium-dependent potassium conductance by increasing extracellular calcium influx or calcium release from intracellular stores. Burke and Sanders (1990) showed that ouabain hyperpolarized canine colonic myocytes and decreased the membrane resistance. The reduction in membrane resistance was the result of an increase in potassium conductance. This increase in potassium conductance was antagonized by TEA and cesium ions (Burke and Sanders, 1990). The data presented here indicate that the hyperpolarization of S neurons induced by low concentrations of ouabain may be mediated through activation of a calcium-dependent potassium channel because the hyperpolarization is sensitive to either TEA or calcium-free medium. However, it is not sensitive to the selective high conductance calcium-activated potassium channel (Maxi-K) blocker iberiotoxin. The ATP-sensitive potassium channel blocker glibenclamide also had no effect on the hyperpolarizations induced by ouabain. It remains to be clarified which subtype of K_{cal} channel is involved in the ouabain-induced hyperpolarization of guinea pig myenteric type S neurons. Thus, data from the literature and the present study indicate that ouabain possesses dual effects on membrane properties and resting membrane potential of several types of cells.

The experiments here do not distinguish between a direct inhibition of calcium-sensitive potassium channels by ouabain or an indirect activation due to elevations in intracellular calcium. Given that removal of extracellular calcium abolishes all signs of hyperpolarization by ouabain, we suggest, as did Higashi et al. (1987), that the effect of ouabain is probably indirect. The focus of this study was not to characterize the specific mechanism of the hyperpolarizing effect of ouabain but rather to identify a method by which to minimize its impact.

The primary effect of ouabain on cell membranes is to inhibit the Na⁺,K⁺ pump, resulting in a depolarization by elimination of the contribution of the electrogenic pump potential to resting membrane potential. In some cells, another effect is to increase potassium conductance by opening potassium channels. The depolarizing and hyperpolarizing effects in myenteric S neurons occur with overlapping concentra-
tions of ouabain and similar time courses. Because these effects antagonize each other, the observed response to ouabain is the net summation of these opposing effects. The masking effect of potassium channel-mediated hyperpolarizations could lead to an underestimation of activity when studying electrogenic Na\textsuperscript{+},K\textsuperscript{+} pump function on cell membrane characteristics using ouabain. In higher concentrations of ouabain, the depolarizing action appears to predominate. For example, in the present study, 5 μM ouabain always produced a net depolarization and never evoked demonstrable hyperpolarizing responses.

These results allowed the selection of optimum conditions for the use of ouabain to determine the possible role of altered electrogenic Na\textsuperscript{+},K\textsuperscript{+} pumping in the resting membrane depolarization of S myenteric neurons that occurs with chronic exposure of guinea pigs to morphine. The 5 μM concentration of ouabain was chosen. Its effect on resting potential was tested in both the presence and absence of TEA in neurons from control animals vs. animals implanted with subcutaneous morphine pellets.

Consistent with previous reports (Leedham et al., 1992), the S neurons from guinea pigs receiving morphine pellets 7 days before the experiment were depolarized by a mean of ~9 mV relative to controls. The application of ouabain (5 μM) to these neurons produced significantly less depolarization of the neurons from the morphine group relative to controls. This is consistent with a conclusion that electrogenic activity of the Na\textsuperscript{+},K\textsuperscript{+} pump contributes less to resting membrane potential in the morphine-treated group than in controls. Indeed, regardless of whether the ouabain was administered in the presence (10.7 mV) or absence (8.5 mV) of TEA, the mean difference in the depolarizing effect of ouabain was similar to the mean difference in resting membrane potential (8.7 mV) between neurons from controls and those from morphine-pretreated guinea pigs. In the presence of 5 μM ouabain, either with or without TEA, the mean membrane potential was nearly identical in S neurons of tolerant and naive guinea pigs, indicating that acute pump inhibition eliminated the difference between them.

Virtually identical results (Gerthoffer et al., 1979) led to the conclusion that adaptive changes in the excitability of smooth muscle cells of the guinea pig vas deferens associated with a resting depolarized state was the result of reduced electrogenic pumping, not of altered diffusion potential. Subsequent biochemical experiments (Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity: Gerthoffer et al., 1979; ouabain binding: Wong et al., 1981; measurements of the α\textsubscript{2} subunit of the Na\textsuperscript{+},K\textsuperscript{+} pump: Hershman et al., 1993, 1995) confirmed a functional decrease in the pump of the smooth muscle cells of the vas deferens. Somewhat parallel studies by Rogers et al. (1993) led to the discovery that adaptive changes in excitability and a depolarized state of canine colonic smooth muscle are associated with a reduction in the messenger RNA of the α\textsubscript{2} subunit of the pump.

The present report provides the first evidence of a connection among adaptive changes in excitability, depolarized resting membrane potential and reduced Na\textsuperscript{+},K\textsuperscript{+} pumping in a neuron. Unfortunately, the myenteric neurons represent a small portion of the mass of smooth muscle and connective tissue in the myenteric plexus/longitudinal muscle preparation. Consequently, biochemical studies of the pump in those neurons are impractical. However, preliminary data in our laboratory indicate that similar relationships exist in the nucleus tractus solitarius (Malanga et al., 1995) and locus ceruleus (Meng et al., 1996) of the guinea pig, areas in which more detailed studies can be conducted. The importance of this association lies in the relevance of the depolarized resting state to opioid tolerance and dependence.

The evidence that the depolarized state of myenteric S neurons from animals chronically exposed to morphine is responsible for the opioid tolerance and dependence in the myenteric plexus/longitudinal muscle preparation is extensive, as discussed by Leedham et al. (1992) and in the review by Fleming and Taylor (1995). Implantation of morphine pellets in guinea pigs induces cross-tolerance to several unrelated hyperpolarizing agents (μ-opioid agonists, α\textsubscript{2} adrenoceptor agonists, 2-chloroadenosine; Leedham et al., 1991; Taylor et al., 1988) without any indication of changes in receptors or signal transduction processes (Leedham et al., 1992). Simultaneously, implantation of morphine pellets induces supersensitivity of the myenteric plexus/longitudinal muscle preparation to a variety of depolarizing agents and procedures, including nicotine, acetylcholine, 5-hydroxytryptamine, potassium and electrical stimulation (Johnson et al., 1978). Administration of naloxone to tolerant preparations in the continued presence of morphine leads to a withdrawal contraction of the longitudinal muscle secondary to a combination of enhanced excitatory synaptic activation and spontaneous firing of S neurons (Johnson et al., 1987). The depolarized state of the neurons ties together all of these effects.

Many procedures that chronically depress cellular activity lead to a compensatory adaptation, which is characterized by supersensitivity to excitatory substances and/or subsensitivity to inhibitory substances (reviews by Fleming et al., 1973 and Fleming, 1976). That tolerance to and dependence on opioids are simply reflections of a general cellular phenomenon of adaptive supersensitivity/subsensitivity has been recognized for many years (Collier, 1966; Goldstein and Shulz, 1977).


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