1999 Torald Sollman Award Lecture

Cellular Adaptation: Journey from Smooth Muscle Cells to Neurons

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ABSTRACT
In the 1960s, it became clear that the adaptation of smooth muscle to denervation was different from that of skeletal muscle. The supersensitivity of denervated smooth muscle extended to agonists unrelated to the lost neurotransmitter and developed on a tissue-dependent time course of several days to several weeks. Several procedures, in addition to denervation, that interrupted excitatory transmission, elicited the phenomenon. The supersensitivity occurred without changes in density or affinity of receptors but correlated with a partial depolarization of the smooth muscle cells. The phenomenon could be mimicked by procedures that acutely depolarized the cells. Electrophysiological, biochemical, and molecular data established that the depolarization was due to reduced electrogenic pumping and reduced density of the Na+,K+ pump. The triggering event for the development of such supersensitivity is not interruption of contact of neurotransmitter with its receptor, but rather the decreased activity of the adapting cells. This is clear from the fact that the inhibitory action of opioids produces similar sensitivity changes in several different populations of guinea pig neurons, including S-type neurons of the myenteric plexus. Subcutaneous implantation of morphine pellets in guinea pigs induces adaptation of S neurons expressed as nonspecific subsensitivity to inhibitory agonists (opioids, α2-adrenoceptor agonists, 2-chloroadenosine) and supersensitivity to excitatory agonists (nicotine, 5-hydroxytryptamine, K+). These changes are accompanied by a partial depolarization of the S neurons and decreased electrogenic Na+,K+ pumping. Chronic implantation of morphine pellets also produces similar nonspecific changes in sensitivity in neurons of the nucleus tractus solitarius and locus ceruleus. It is suggested that depressed activity of these neurons leads to an electrophysiological adaptation, presumably due to reduced density of Na+,K+ pump proteins, as demonstrated in smooth muscle.

With Appreciation
It is a great honor to be chosen by the American Society of Pharmacology and Experimental Therapeutics, which has played such a significant role in my professional life, for the Torald Sollman Award. My sincere thanks go to Wyeth-Ayerst Pharmaceutical Company for their support for this award. At a time such as this, one reflects upon the individuals who have had a particularly great impact on one's career. I have identified seven individuals to whom I am particularly indebted. My postdoctoral mentors were Ullrich Trendelenburg and Otto Krayer. Trendelenburg, a pioneer in quantitative pharmacology was, and has been throughout my career, the greatest inspiration scientifically. Otto Krayer, a fine scientist and a great leader, put together a remarkable group of pharmacologists at Harvard Medical School in the 1950s. Postdoctoral fellows were treated as an integral part of that outstanding group, creating in them a strong sense of belonging. Daniel T. Watts recruited me to West Virginia in 1960. With a great ability to develop young faculty, Watts was another significant role model as a chair. In my 33 years as a chair, I have been fortunate, at different times, to recruit back as faculty members, two exceptional young scientists who had received their Ph.D.s in my laboratory. Their talents, friendship, and collaboration had enormous impact on most of the work about to be described. I refer to David P.
Westfall, currently Vice President for Academic Affairs, University of Nevada, Reno, and David A. Taylor, currently Professor of Pharmacology at West Virginia University. The friendship and willingness of Robert E. Stitzel and Charles R. Craig to share the administrative load of the department chair made it possible for me to function effectively as a laboratory scientist. A number of excellent students, postdoctoral associates, and faculty collaborators (see Acknowledgments) contributed enormously. Unfortunately, time and space allow tracing only one theme, admittedly a dominant one, from the laboratory, leaving much exciting work unmentioned. Choosing this one over much interesting work on cardiac pharmacology and hypertension was not easy! Finally, I acknowledge the encouragement, trust, and confidence provided by my best friend for 50 years, my wife Dolores. She and our terrifically supportive children are part of this.

Introduction

In the 1950s and 1960s, quantitative pharmacology was rapidly developing. There had been a new surge in receptor theory with the work of Stephenson (1956) and Ariëns (1964). The development of supersensitivity following denervation was a topic of high interest, particularly spurred by the classical work of Thesleff (1960), who presented evidence that denervation of skeletal muscle induced a spread of receptors outward from the endplate, explaining the marked supersensitivity to nicotinic agonists. Work in the laboratories of Krayer and Trendelenburg brought direct experience for me in receptor theory (Fleming and Hawkins, 1960) and the appropriate use of dose-response curves to study sensitivity (Fleming and Trendelenburg, 1961; Trendelenburg et al., 1962a,b). By the time that I began my first faculty appointment at West Virginia, those lessons had become the core of my approach to pharmacology.

Characteristics of Supersensitivity of Smooth Muscle

Early work indicated that the mechanism of denervation supersensitivity in smooth muscle was unlikely to be the result of changes in a specific receptor, in contrast to Thesleff’s (1960) findings in skeletal muscle. Interrupting the excitatory, noradrenergic innervation of the smooth muscle of the cat nictitating membrane, produced a nonspecific supersensitivity. Such denervation produced equivalent increases in sensitivity to the stimulatory effects of α-adrenergic agonists, muscarinic agonists, and ions such as barium (Fleming, 1963; Morrison and Fleming, 1971).

Other procedures, including preganglionic denervation (decentralization) and chronic depletion of norepinephrine with reserpine also produced nonspecific supersensitivity of the nictitating membrane (Fleming, 1963). The shift to the left of the dose-response curve for norepinephrine was similar for decentralization and chronic administration of reserpine (Fleming, 1963). The time courses of the development of supersensitivity were the same for postganglionic denervation, decentralization, and chronic transmitter depletion. However, the shift of the norepinephrine curve was greater when induced by postganglionic denervation (henceforth simply called denervation) than by decentralization or chronic reserpine (Fleming, 1963). During this same period, Trendelenburg had established that denervation produced supersensitivity via two distinct and independent processes (for review, see Trendelenburg, 1963, 1966). One mechanism, specific for substrates of the neuronal transport system for norepinephrine, is associated with the loss of that transport system. This mechanism is mimicked by cocaine, which inhibits the neuronal transport system. The other mechanism, due to adaptation of the postsynaptic cells, is nonspecific. Trendelenburg (1966) introduced the terms presynaptic and postsynaptic supersensitivity to distinguish between these mechanisms. Because they do not alter neuronal uptake, decentralization and chronic reserpine induce only the postsynaptic variety. Denervation, because it leads to degeneration of the nerve endings, produces both forms of supersensitivity, which are additive and, therefore, produce a greater shift in the dose-response curve for norepinephrine than for agonists that are not substrates for neuronal uptake.

The above-mentioned concepts predict that, for agonists that are not substrates for neuronal uptake, the shift in the dose-response curve would be the same after either denervation or decentralization. This prediction was corroborated for barium (Morrison and Fleming, 1971).

The existence of these two types of supersensitivity has been established in a number of noradrenergically innervated muscles and the suggestion was made that the two mechanisms be identified by new terms, deviation supersensitivity and adaptive supersensitivity (Fleming, 1975). In a review by Fleming et al. (1973), the characteristics and procedures for inducing supersensitivity in many peripheral tissues were summarized. It was clear that interruption of excitatory neurotransmission, by any means, produced nonspecific supersensitivity of many smooth muscles to excitatory agonists. The muscles differed in the time course of the appearance of adaptive supersensitivity and in its magnitude, but otherwise they were remarkably similar to the nictitating membrane. The nonspecific nature of the supersensitivity suggested at an early stage that the phenomenon in smooth muscle could not be explained by changes in a specific population of receptors (Hudgins and Fleming, 1966). Proof of that concept had to wait for the development of receptor-ligand binding techniques. The advent of that methodology clearly established that adaptive supersensitivity occurred without changes in \( B_{\text{max}} \) or \( K_D \) values in smooth muscle of the guinea pig vas deferens (Cowan et al., 1985), rat vas deferens (Abel et al., 1985), or rat caudal artery (Nasseri et al., 1985).

Mechanism of Adaptive Supersensitivity in Smooth Muscle

The nonspecific nature of adaptive supersensitivity in smooth muscle led our laboratory to the hypothesis that the mechanism might be electrophysiological, rather than related to changes in receptors or transduction processes. Subsequent experiments with intracellular recording in the smooth muscle of the guinea pig vas deferens strongly supported that hypothesis. Denervation, decentralization, chemical destruction of noradrenergic neurons with 6-hydroxydopamine, and depletion with reserpine each induces a nonspecific supersensitivity to a variety of unrelated agonists (Westfall, 1970; Westfall et al., 1972; Fleming and Westfall, 1975). There was no supersensitivity 2 days after interrup-
tion of transmission, but it was fully developed on the 4th day and remained constant for many days thereafter. Furthermore, the fact that the vas deferens was quiescent until stimulated, made it ideal for intracellular electrical recording.

The first paper on the relevant intracellular electrical recording was by Fleming and Westfall (1975). The data in Table 1, derived from that paper, demonstrate that both denervated and decentralized cells were significantly depolarized by 8 to 10 mV at 7 days after the surgical procedure, a time when supersensitivity was fully developed. Table 1 also presents a time course in the decentralized preparations. Resting membrane potential was unchanged 2 days after the operation, whereas the decentralized cells were depolarized by 8 to 9 mV from 4 days through 12 to 17 days. Thus, the time course was identical with that of the supersensitivity.

To propose that the slowly developing partial depolarization is responsible for the slowly developing, nonspecific supersensitivity required two additional hypotheses. First, the depolarization must bring the resting membrane potential closer to the threshold potential for cellular excitation. Second, procedures that acutely depolarize the cells must induce acute, nonspecific supersensitivity. The first hypothesis was tested by Goto et al. (1978). With externally applied depolarizing currents and by determining the membrane potential at which action potentials were generated, Goto et al. (1978) established that the threshold potential was virtually identical in control (40.4 ± 2.2 mV) and 7-day denervated (39.0 ± 2.8 mV) cells of the guinea pig vas deferens.

There are two components that additively contribute to resting membrane potential (for review, see Fleming, 1980, for an analysis of these components in smooth muscle). One component is the diffusion potential, determined by the unequal distribution of, and permeabilities to, ions across the membrane. The outward diffusion of K⁺ is the central factor in the diffusion potential. The other component of resting membrane potential is the electrogenic activity of the Na⁺,K⁺ pump. This pump, which functions to maintain the diffusion potential over time by transporting Na⁺ out in exchange for K⁺ in, as the ions go down their concentration gradients, does not transport these two ions at equal rates. Rather, it exchanges three Na⁺ for two K⁺, producing a net outward positive current. This current, moving across the resistance of the membrane, contributes directly to the negative potential on the inside of the membrane.

With knowledge of these two factors in resting membrane potential, Urquilla et al. (1978) investigated the second above-mentioned hypothesis. For example, raising the K⁺ concentration in the extracellular environment (i.e., in an organ-bathing solution) depolarized the cells, in a concentration-dependent manner, by altering the diffusion potential. However, inhibiting the Na⁺,K⁺ pump with agents such as ouabain produces a partial depolarization by acutely inhibiting the electrogenic pump component. Urquilla et al. (1978) demonstrated that doubling the normal concentration of K⁺, or adding 10 μM ouabain to the bathing medium of the guinea pig vas deferens, acutely depolarized the cells by 8 to 10 mV and induced supersensitivity to histamine and to adrenoceptor agonists (norepinephrine and methoxamine) by a factor of 3- to 4-fold, thus closely mimicking the chronic effects of denervation or decentralization. There seemed little doubt that the depolarization caused the supersensitivity. High potassium and pump inhibition could conceivably alter neuronal uptake. However, because the changes in sensitivity were not different for norepinephrine (a substrate for neuronal uptake) and for methoxamine (not a substrate), which activate the same receptors (α₁), it can be concluded that neuronal uptake was not significantly altered.

The next question was, by what mechanism does interruption of the innervation produce depolarization? The first candidate chosen to be examined was the electrogenic Na⁺,K⁺ pump, a fortunate choice. Experiments by Goto and Gerthoffer came together in a key publication (Gerthoffer et al., 1979). Electrophysiological experiments established that 10 μM ouabain (the optimal concentration for pump inhibition) acutely depolarized control cells of the vas deferens by 8 to 13 mV (Urquilla et al.; 1978; Gerthoffer et al., 1979). However, ouabain had no significant effect on the resting potential of chronically denervated cells. These results indicated that denervation had already selectively reduced the electrogenic pump potential to a virtually immeasurable level. The results further indicated that the diffusion potential was essentially unchanged by denervation because in the presence of pump inhibition, the membrane potential was similar in control and denervated cells.

Gerthoffer’s contribution to Gerthoffer et al. (1979) was biochemical analysis of the activity of Na⁺,K⁺ ATPase, the enzymatic equivalent of the Na⁺,K⁺ pump. It was established that chronic denervation, decentralization, and transmitter depletion (reserpine) each significantly reduced the maximum rate of hydrolysis of ATP by the enzyme in homogenates of the guinea pig vas deferens. A time course study indicated that the decrease occurred between day 2 and day 4 after the operation, as did the appearance of supersensitivity and depolarization. Thus, the evidence strongly suggested

### Table 1

<table>
<thead>
<tr>
<th>D Postop</th>
<th>Days Postoperative</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>12–17</th>
</tr>
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<tbody>
<tr>
<td>Control&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-58.7 ± 0.9 (28.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-48.2 ± 1.3 (20.7)</td>
<td>-63.2 ± 2.0 (24.13)</td>
<td>-54.7 ± 1.5 (29.13)</td>
<td>-50.6 ± 1.1 (29.6)</td>
</tr>
<tr>
<td>Denervated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-59.2 ± 2.4 (9.5)</td>
<td>-59.6 ± 1.7 (14.4)</td>
<td>-59.6 ± 1.5 (24.6)</td>
<td>-50.6 ± 1.1 (29.6)</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>10.5 mV&lt;sup&gt;**&lt;/sup&gt;</td>
<td>7.9 mV&lt;sup&gt;*&lt;/sup&gt;</td>
<td>8.5 mV&lt;sup&gt;**&lt;/sup&gt;</td>
<td>9.0 mV&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Decentralized&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-59.9 ± 2.4 (11.5)</td>
<td>-51.7 ± 1.7 (18.4)</td>
<td>-54.7 ± 1.5 (29.13)</td>
<td>-50.6 ± 1.1 (29.6)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Data taken from Fleming and Westfall (1975).
<sup>b</sup> All comparisons were made in paired vasa deferentia, one side operated, the other control.
<sup>*</sup> Numbers in parenthesis represent number of cells, number of vasa deferentia.
<sup>p < .01; ** p < .001</sup>
that the depolarization was the result of decreased activity of the Na\(^+\),K\(^+\) ATPase.

The next issue was whether the decreased activity of the Na\(^+\),K\(^+\) ATPase was the consequence of a qualitative change in pump function or a decrease in available pump protein. Two lines of evidence indicated that the latter was correct. Ouabain-binding experiments (Wong et al., 1981) demonstrated that chronic denervation, decentralization, or reserpine significantly decreased specific ouabain binding by 30 to 40%. The time course was consistent with the depolarization.

Definitive measures of pump protein abundance were obtained by Ken Hershman. The Na\(^+\),K\(^+\) pump has two subunits, \(\alpha\) and \(\beta\) (Swedner, 1989). The \(\alpha\)-subunit possesses the binding sites for Na\(^+\),K\(^+\), and cardiac glycosides and the ATPase activity. There are three isoforms of the \(\alpha\)-subunit, \(\alpha_1\), \(\alpha_2\), \(\alpha_3\). Which isoform predominates varies from one cell type to another. With a combination of specific antibodies and chemiluminescence, Hershman et al. (1993a) developed a very sensitive slot blot analysis of the \(\alpha\)-subunit isoforms. The primary isoform in the smooth muscle of the vas deferens was the \(\alpha_2\). Hershman et al. (1993b, 1995) established that there was a 40% reduction in the \(\alpha_2\)-isoform in vasa deferentia from guinea pigs chronically depleted of norepinephrine that followed a similar time course as the depolarization and the supersensitivity. Thus, all of the data came together to establish that the supersensitivity was directly the result of a partial depolarization, itself the consequence of a reduced abundance of Na\(^+\),K\(^+\) pump protein.

The association of supersensitivity, partial depolarization, and reduced electrogenic pumping in smooth muscle is not restricted to the guinea pig vas deferens. Abel et al. (1981) used chronic administration of reserpine to induce supersensitivity in the rabbit saphenous artery. Reserpine (0.3 mg/day) produced virtually complete depARATION of norepinephrine in the artery within 24 h. Treatment for 3 or 7 days, but not for only 1 day, produced supersensitivity to the stimulatory effects of norepinephrine, methoxamine, histamine, acetyl-choline, and potassium.

The control membrane potential (Em) of saphenous artery smooth muscle cells was 65.2 ± 0.8 mV. After 3 days of pretreatment of the rabbits with reserpine, the mean Em was −60.3 ± 0.9 mV, a statistically significant depolarization of 5 mV. The two groups of cells did not differ in the potential at which contractions were activated, equivalent to threshold. Furthermore, ouabain (3 \(\mu\)M) depolarized the control cells, but not the cells from the 3-day reserpine-pretreated animals, resulting in equal Em values in the two groups. Thus, the phenomenon exists in different types of smooth muscle in different species.

**Adaptive Super- and Subsensitivity as Expressed in Tolerance to Opioids**

The myenteric plexus is a collection of ganglia lying between the longitudinal and circular layers of smooth muscle of the ileum. The physiology and morphology of those neurons are extensively discussed in the book by Furness and Costa (1987). With careful dissection of a strip of longitudinal smooth muscle, the myenteric plexus continues to adhere to the muscle. The longitudinal muscle/myenteric plexus (LM/MP) preparation can be used for a variety of pharmacological and electrophysiological experiments. The ganglia include the cell bodies of the postganglionic, cholinergic axons that innervate the smooth muscle. Transmural electrical stimulation causes these neurons to release acetylcholine, which in turn induces contraction of the muscle (Paton, 1957). Morphine acts upon opioid receptors to inhibit this release (Kosterlitz et al., 1972). Adequately spaced single transmural electrical shocks produce a regular series of “twitch” contractions of the muscle and opioids inhibit the twitch in a concentration-dependent manner, due entirely to their actions on the neurons.

Goldstein and his associates (Schulz and Goldstein, 1973) used this preparation, in conjunction with the s.c. implantation of pellets of morphine in guinea pigs, to demonstrate tolerance to morphine. Schulz and Goldstein (1973) reported that, in LM/MP preparations taken from guinea pigs chronically implanted with morphine pellets, not only were the preparations tolerant (subsensitive) to the inhibitory effects of morphine, but they were supersensitive to the stimulatory effects of 5-hydroxytryptamine (5-HT). Responsiveness of the smooth muscle, per se, to drugs was unchanged. Based on the work with the guinea pig vas deferens, it occurred to us that the results of Schulz and Goldstein (1973) might be explained by an adaptive partial depolarization of the myenteric neurons. Such a phenomenon had never been described in neurons, but never had attempts to seek it out been published.

If the adaptive changes in the myenteric neurons were due to a partial depolarization, it was hypothesized that the LM/MP would be supersensitive to other excitatory substances in addition to 5-HT and subsensitive to inhibitory substances in addition to opioids. Both predictions were experimentally proven. Guinea pigs were implanted either with four pellets of morphine (75 mg/pellet) or four placebo pellets. Seven days later, the LM/MP preparations were set up. Johnson et al. (1978) found that, in the preparations from the morphine-pretreated guinea pigs, the concentration-response curves for 5-HT, nicotine, and potassium were all significantly shifted to the left, indicating supersensitivity. Experiments to separate the effects of potassium on the nerves from its effects on the muscle established that the supersensitivity resided solely in the neurons.

Some years intervened until funding could be obtained to pursue this novel approach to opioid tolerance. Ten years later, Taylor et al. (1988) established that LM/MP preparations were characterized by shifts to the right (subsensitivity) of concentration-response curves of three separate classes of inhibitory agonists (morphine, \(\alpha_2\)-adrenoceptor agonists, and 2-chloroadenosine).

Two types of neuron are identifiable electrophysiologically in the myenteric plexus (Nishi and North, 1973; Hirst et al., 1974). Hirst et al. (1974) named these neurons S and AH. The excitatory nerves to the longitudinal muscle are S neurons, whereas the AH neurons are apparently sensory and innervate other neurons in the plexus (Furness and Costa, 1987; Hendriks et al., 1990). The primary functional opioid receptors on myenteric neurons are \(\mu\)- and \(\kappa\)-receptors and are restricted to S neurons. Consistent with the membrane-potential hypothesis of tolerance to morphine, the excitatory agonists to which chronic morphine induces supersensitivity (nicotine, 5-HT, K\(^+\)) have their acute effects on S neurons by depolarization. Conversely, the inhibitory agonists, to which chronic morphine induces subsensitivity (morphine, \(\alpha_2\)-adrenoceptor agonists, and 2-chloroadenosine) inhibit S neurons.
Electrophysiological Investigation of Opioid Tolerance in Guinea Pig

Experiments were done with intracellular recording electrodes in myenteric S neurons from guinea pigs implanted 7 days previously with either morphine or placebo pellets. Because S neurons have opioid receptors and AH neurons do not, it was not predicted that chronic exposure to morphine would induce an adaptation only in S neurons. As expected, morphine pellet implantation caused a significant 7- to 9-mV hyperpolarization in S neurons but not AH neurons (Leedham et al., 1992). In one series of experiments (Leedham et al., 1992), resting membrane potential and the threshold potential for firing action potentials were both measured in placebo and morphine-tolerant S neurons. In that series, the neurons from morphine pellet-implanted animals were significantly depolarized by a mean of 8.7 mV, whereas the small difference in threshold (2.3 mV) was not significant. Thus, as predicted, the S neurons from tolerant animals were partially depolarized, such that the difference between resting potential and threshold was reduced.

The next corollary hypothesis was that, if the subsensitivity of hyperpolarizing agents for inhibiting release of transmitter were merely the result of resetting the set point (resting membrane potential), the magnitude of the hyperpolarizing effect of these agents would not be changed in individual S neurons from tolerant preparations. One drug from each class of inhibitory agonists was chosen: morphine (0.1 µM), clonidine (0.3 µM), and 2-chloroadenosine (0.1 µM). These concentrations were selected because, in the LM/MP twitch experiments, these concentrations inhibited the twitch ~70% in control preparations, but ~10% in tolerant preparations, thus clearly demonstrating the phenomenon of tolerance (subsensitivity). Table 2 presents the results (Meng et al., 1997). As predicted, the cells from the tolerant group yielded hyperpolarizing responses not different from control. These data further supported the conclusion that the tolerance is not due to a decrease in the density of receptors or to a decrease in the transduction processes, but rather, to an alteration in the set point, i.e., resting potential. Receptor-mediated or transduction changes would only be compatible with significantly decreased hyperpolarizing effects of the agonists.

The final electrophysiological question for the myenteric neurons was whether the adaptive partial depolarization reflected a change in electrogenic Na⁺, K⁺ pumping or in the diffusion potential. The work of Kong et al. (1997) provided a clear answer. The results are summarized in Table 3. The tolerant cells were already partially depolarized, as previously established. Ouabain (5 µM) depolarized control cells twice as much as tolerant cells, suggesting that the tolerant cells had a lesser electrogenic pump potential. This conclusion is reinforced by the fact that the Em values of the two groups of cells in the presence of ouabain were virtually identical, suggesting that the diffusion potentials did not differ.

To determine whether other guinea pig neurons adapt to opioids in a similar manner, brain slices containing either the nucleus tractus solitarius (nTS) or the locus ceruleus (LC) were studied electrophysiologically. Slices were taken from appropriate brain regions of guinea pigs implanted 7 days previously with pellets containing either morphine or placebo. Because the slice experiments required younger, smaller animals, the guinea pigs each received only two pellets. The sensitivity to agonists was determined by inhibition of spontaneous firing of action potentials, measured with extracellular electrical recording.

In the nTS, concentrations of morphine (1 µM), muscimol (0.3 µM), 2-chloroadenosine (1 µM), and clonidine (0.1 µM) that inhibited firing rates by ~60% in control slices, inhibited firing rates by only ~20% in slices from animals implanted with morphine pellets, indicating a nonspecific subsensitivity (Malanga et al. 1997). Full concentration-response curves were determined with muscimol, indicating that the chronic exposure to morphine produced a 5.6-fold decrease in sensitivity to that nonopioid γ-aminobutyric acid-receptor agonist. However, the excitatory effect of K⁺ (7.3 mM) was significantly increased. Thus, the pharmacological profile was consistent with an electrophysiological adaptation, rather than a change in receptors or signal transduction.

Similar methods applied to pontine slices established that morphine pellets induced a nonspecific subsensitivity of LC neurons to morphine, muscimol, and clonidine (Meng et al., 1996). Most recently, whole-cell patch clamp experiments in pontine slices (Kong et al., 1999) have identified a significant

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**TABLE 2**

Hyperpolarizing responses to agonists in myenteric S neurons from guinea pigs implanted for 7 days with placebo or morphine pellets

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Resting Em (mV)</th>
<th>Hyperpolarizing Effect (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Morphine</td>
</tr>
<tr>
<td>Placebo</td>
<td>-46.8 ± 0.8</td>
<td>6.6 ± 0.5</td>
</tr>
<tr>
<td>Morphine</td>
<td>-39.6 ± 1.5*</td>
<td>5.8 ± 0.8</td>
</tr>
</tbody>
</table>

* Data taken from Meng et al. (1997).

a N values were 18 placebo cells and 28 cells from guinea pigs implanted with morphine pellets. Morphine was tested on all cells and the other drugs on subsets of seven or more cells from this total group.

* p < .05.
partial depolarization (mean of 8.4 mV) in LC neurons from guinea pigs implanted with morphine compared with placebo. There was no difference in cellular input resistance, suggesting that the depolarization does not involve a change in diffusion potential.

Research currently underway is aimed at quantifying subunit isoforms of the Na\(^{+}\),K\(^{+}\) pump in myenteric nTS, and LC neurons. Methods include both slot blot analyses with antibodies and chemiluminescence and immunohistochemistry. The data obtained will complete the comparison between denervated smooth muscle and opioid-tolerant neurons. At present, the data support the conclusion that, in both cell types, the adaptive sensitivity changes are the result of a partial depolarization of the cell membrane, which is, in turn, the consequence of a quantitative reduction in electrogenic Na\(^{+}\),K\(^{+}\) pumping. In the smooth muscle of the guinea pig vas deferens, the reduced activity is due to a marked reduction in the quantity of pump protein. Ongoing experiments will indicate if the same is true of guinea pig neurons made tolerant to opioids.

Clear evidence has been presented by other laboratories that opioid tolerance in the rat locus ceruleus is specific and related to a change in the transduction system of opioid receptor activation (Nestler, 1992). However, those results are in a different species and with different methods of induction of tolerance. It must be recognized that different mechanisms may contribute to tolerance. There can be little doubt that one of those mechanisms involves changes in the Na\(^{+}\),K\(^{+}\) pump. The simultaneous development of subsensitivity to inhibitory agonists and transmitters and supersensitivity to excitatory agonists and transmitters can readily explain both tolerance to opioids and dependence/withdrawal reactions to opioid use.

The concept is based upon the reasonable assumption that inhibition of neuronal firing is a function of the total membrane potential achieved, i.e., resting potential plus the hyperpolarizing effect of the drug (Fig. 1). If the resting potential is less than “normal” and the hyperpolarizing effect and threshold for firing are unaltered, the sum will be less. Therefore, tolerance to the hyperpolarizing drug exists because a greater hyperpolarizing effect, i.e., more drug, is necessary to compensate for the depolarized state of the membrane. Dependence would exist because the presence of the hyperpolarizing drug (opioid) is somewhat compensating for the partially depolarized state. The removal of the opioid would allow the depolarized state to appear with enhanced sensitivity to natural excitatory transmitters.

Table 3: Depolarization induced by ouabain (5 μM) in myenteric S neurons from guinea pigs implanted for 7 days with placebo or morphine pellets

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Resting Em</th>
<th>Ouabain-Induced Depolarization</th>
<th>Em with Ouabain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo(a)</td>
<td>-54.0 ± 1.8</td>
<td>15.9 ± 2.7</td>
<td>-38.1</td>
</tr>
<tr>
<td>Morphine(b)</td>
<td>-45.3 ± 2.2</td>
<td>7.4 ± 3.3(a)</td>
<td>-37.9</td>
</tr>
</tbody>
</table>

\(a\) Data taken from Kong et al. (1997).
\(b\) Resting Em minus depolarizing effect of ouabain.

Threshold does not alter the density or properties of alpha-1 adrenergic receptors in rat vas deferens. [J Pharmacol Exp Ther 235:570–577.]

Table 3: Normal S neuron vs. Tolerant S neuron

<table>
<thead>
<tr>
<th>Em (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>40</td>
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</tbody>
</table>

Fig. 1. Relationship of resting potential, threshold, and agonist effect to inhibit or excite representative myenteric S neurons. Threshold and resting Em are based upon published data (Leedham et al., 1992). Threshold (−20 mV) is the same in both groups, whereas resting Em (−50 and −42 mV, respectively) represents a depolarized state in tolerant neurons. The −58-mV line represents a hypothetical level of Em necessary for a given amount of inhibition. It is clear that a larger hyperpolarization (16 versus 8 mV) is required to inhibit the tolerant neuron and less excitatory depolarization (22 versus 30 mV) is required to activate the tolerant neuron.

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