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Synaptic Modulation by Dopamine of Calcium Currents in Rat Pars Intermedia

Peter J. Williams, Brian A. MacVicar, and Quentin J. Pittman

Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Melanotrophs of the rat pars intermedia are innervated by dopaminergic fibers traveling through the pituitary stalk which inhibit secretion via an action on D-2 receptors. As secretion from the melanotroph has been shown to be calcium (Ca^{2+}) dependent, it is possible that dopamine may have an action to inhibit Ca^{2+} currents in these cells. This possibility was tested by examining the effects of exogenously applied dopaminergic agonists or synaptically released dopamine upon Ca^{2+} currents recorded under single electrode voltage clamp in intact rat pars intermedia *in vitro*.

Following blockade of sodium and potassium currents in melanotrophs, Ca^{2+} spikes were elicited with intracellular injection of depolarizing currents; electrical stimulation of the pituitary stalk caused an inhibition of the Ca^{2+}-based action potentials which lasted for several seconds. Using single-electrode voltage-clamp techniques, we recorded inward Ca^{2+} currents corresponding to the T, N, and L types (see Williams et al., 1990). Stimulation of the pituitary stalk inhibited both the low- and high-threshold peak inward Ca^{2+} currents elicited from a holding potential of −90 mV. In contrast, when noninactivating Ca^{2+} currents were elicited from a holding potential of −30 mV, the currents were not altered by stalk stimulation. This pattern of inhibition of the Ca^{2+} currents was consistent with the preferential inhibition, by stalk stimulation, of the N and T Ca^{2+} currents, while sparing the L current.

We observed that inhibition of Ca^{2+} currents due to stalk stimulation was completely reversed by bath perfusion of domperidone (1 μM), an antagonist of dopamine at the D-2 receptor. Quinpirole, a D-2 receptor agonist, mimicked the action of pituitary stalk stimulation by inhibiting Ca^{2+} currents elicited from a holding potential of −90 mV, but not from −30 mV.

To examine the role of G-proteins in mediating dopaminergic inhibition of Ca^{2+} currents in the melanotrophs, rats were pretreated with pertussis toxin, which blocks the actions of some G-proteins. In pertussis toxin-treated rats, only 25% of cells showed any inhibition of Ca^{2+} currents due to stalk stimulation compared with 100% of controls. In another series of experiments, cells were impaled with electrodes containing GTPγS, a GTP analog which leads to irreversible activation of G-proteins. In these cells, only the noninactivating Ca^{2+} current was present, indicating that the transient currents normally inhibited by dopamine were already maximally inhibited by GTPγS.

These experiments thus indicate that both exogenously applied D-2 agonist and synaptically released dopamine act at a D-2 receptor on the melanotroph to inhibit certain Ca^{2+} currents via a G-protein mediated mechanism. This is the first characterization of synaptic modulation, by dopamine, of Ca^{2+} currents in the mammalian nervous system.

The melanotroph of the pars intermedia is a neuroendocrine cell which synthesizes and releases α-melanocyte-stimulating hormone (αMSH) and several peptides of the endorphin family (Goldman et al., 1983). These cells show many of the ionic currents typical of neurons; in particular, they generate action potentials comprising both sodium (Na^+) and calcium (Ca^{2+}) components (Douglas and Taraskevich, 1980). Ca^{2+} is required for secretion from these cells (Taraskevich et al., 1986).

Melanotrophs are known to be under inhibitory control mediated via dopaminergic fibers which arise in the hypothalamus, travel through the pituitary stalk, and synapse directly on the melanotrophs (Baumgarten et al., 1972; Howe, 1973). Dopamine (DA) appears to act on a population of D-2 receptors on the melanotroph, which, when activated, reduce action potential frequency (Douglas and Taraskevich, 1978, 1982) and depress hormone release (Tilders et al., 1975; Cote et al., 1985). We have described an action of synaptically released DA at this synapse which hyperpolarizes the cell through an increase in potassium (K^+) conductance (Williams et al., 1989a). As it is now well established that a number of inhibitory transmitters, including DA, can inhibit voltage-sensitive Ca^{2+} channels (reviewed by Miller, 1987; Tsien et al., 1988), we hypothesize that DA may have an additional action at the melanotroph to inhibit Ca^{2+} currents. We (Williams et al., 1990) and others (Taleb et al., 1986) have previously described 3 different Ca^{2+} currents in the melanotroph which appear to correspond to the T, N, and L types described in a number of other neuronal types (Nowycky et al., 1985; McCleskey et al., 1986). In the present experiments, we have carried out single-electrode voltage-clamp recordings in melanotrophs under conditions allowing the isolation and measurement of Ca^{2+} currents in order to identify the action of synaptically released DA and exogenously applied DA agonists on these currents. We have also investigated the participation of G-proteins in the modulation of Ca^{2+} currents in the melanotroph.

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Correspondence should be addressed to Peter J. Williams, Medical Physiology, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, Canada T2N 4N1.

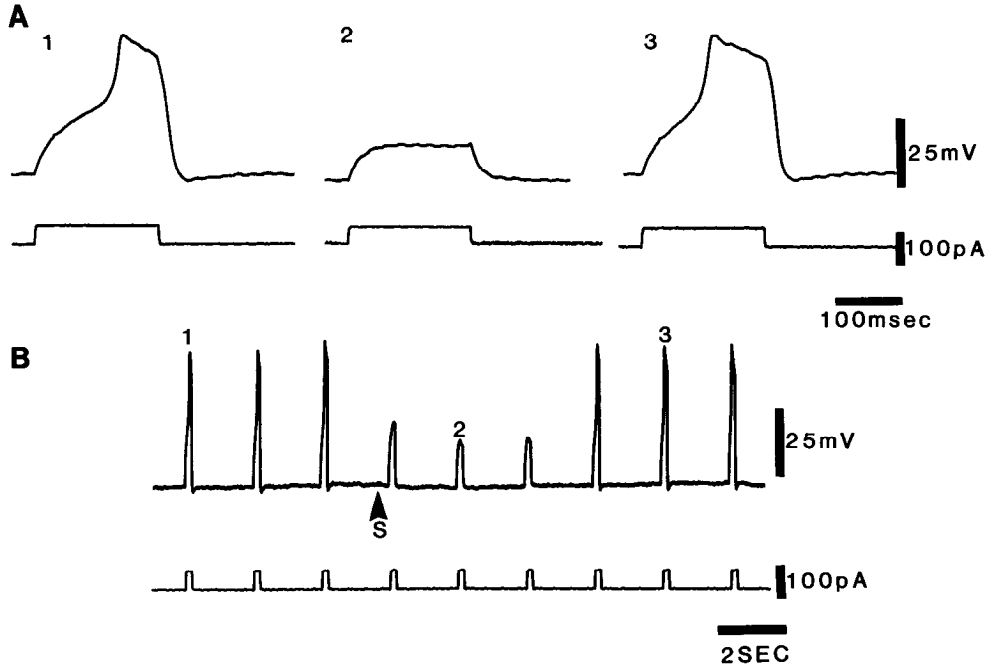

Figure 1. *A*, Stalk stimulation inhibits calcium spikes. Ca^{2+} spikes (*upper*) in response to intracellular injection of depolarizing current (*lower*) in a melanotroph in the intact isolated pituitary. Calcium-based action potentials are shown before (*1*), approximately 2 sec after (*2*) and following recovery (*3*) from stimulation of the pituitary stalk (10 pulses at 50 Hz). *B*, A slower time trace of the experiment described in *A* indicating the points from which the voltage traces were taken and the time at which the stalk (*S*) was stimulated from a bipolar electrode positioned on the stalk. This experiment and all subsequent experiments from which the figures in this paper were obtained were carried out in the presence of 100 μM bicuculline and 0.5 mM 4-AP in the perfusate. The intracellular electrode contained QX-222 to block Na^+ spikes and TEA and Cs^+ acetate to block K^+ conductances.

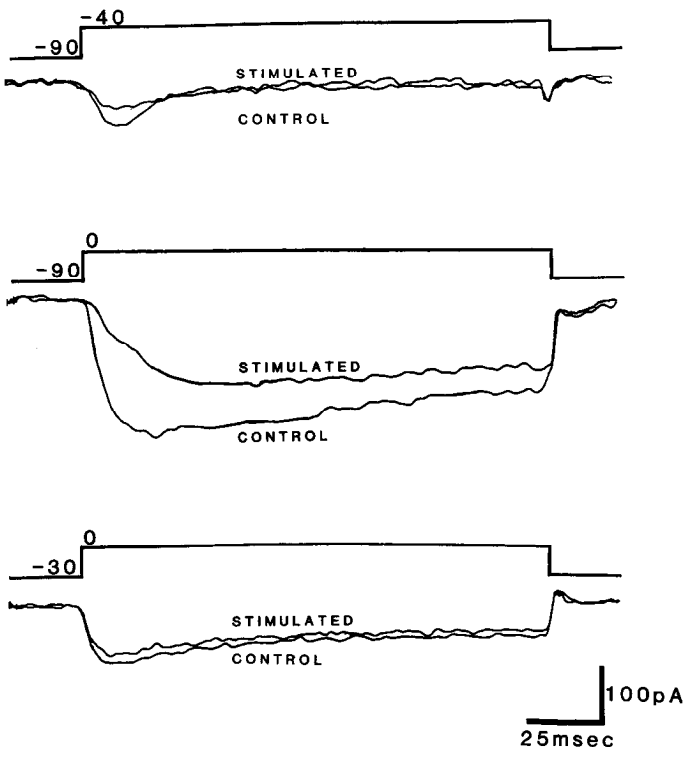

Figure 2. Stalk stimulation inhibits calcium currents evoked from a −90 mV holding potential, but not from −30 mV. Voltage-clamp current recordings from a melanotroph, wherein the voltage was clamped to the holding potentials indicated to the *left* of each voltage trace and then stepped up to the membrane potentials indicated above each voltage trace. Current traces are indicated below each voltage trace and are taken before (*Control*) and after (*Stimulated*) stimulation of the pituitary stalk (10 V, 1 msec, single pulse). Cobalt-insensitive leak currents have been subtracted from this and all subsequent figures.

Materials and Methods

Experimental procedures for maintenance of pituitaries *in vitro*, as well as isolation and measurement of Ca^{2+} currents, were identical to those described in the preceding paper (Williams et al., 1990). Of particular relevance to the present experiments is the fact that Na^+ currents are inhibited by intracellular injection of a Na^+ channel blocker, QX-222, thus avoiding the need to add TTX to the perfusion fluid and allowing us to stimulate afferents to the melanotrophs. Ca^{2+} currents and spikes were isolated by impaling cells with microelectrodes (80–100 MΩ) containing QX-222 (1 mM), cesium (Cs^+) acetate (0.5 M), and TEA acetate (50 mM) to block Na^+ and K^+ currents. The extracellular medium contained 4-aminopyridine (4-AP) to ensure complete block of melanotroph K^+ currents. Afferent stimulation was accomplished with a bipolar silver stimulating electrode positioned on the cut end of the pituitary stalk approximately 3 mm from the recording site in the intermediate lobe. All experiments were carried out in the presence of 100 μM bicuculline to block a $GABA_A$-mediated chloride conductance which can also be activated following stalk stimulation (MacVicar and Pittman, 1986; Williams et al., 1989b). Using a multiple-valve system, D-2 agonists or antagonists of known concentrations were introduced into the recording chamber without disrupting the flow of perfusate. To evaluate the potential role of G-proteins, rats were pretreated with pertussis toxin as previously described (Aghajanian and Wang, 1986; Colmers and Pittman, 1989). Briefly, rats were anesthetized with sodium pentobarbital and, under stereotaxic control, given an injection of 1.5 μg pertussis toxin in 15 μl artificial cerebrospinal fluid into a lateral cerebral ventricle (Pittman et al., 1985). They were allowed to recover for 3–4 d before pituitaries were taken for electrophysiological studies.

Bicuculline methiodide, 4-AP, and tetraethylammonium chloride (TEA) were obtained from Sigma (St. Louis); all other salts were obtained from Fisher Scientific (Fair Lawn, NJ). Pertussis toxin was purchased from List Laboratories, and GTPγS from Boehringer-Mannheim. Domperidone, quinpirole, and QX-222 were respective gifts of Janssen Pharmaceuticals, Eli Lilly Laboratories, and Astra Pharmaceuticals. Drugs were stored as stock solutions and protected from light until they were dissolved in the perfusate immediately before use.

Results

Intracellular recordings were obtained from melanotrophs that displayed resting potentials of −40 mV or more and input resistances of >400 MΩ. Action potentials could be elicited in all

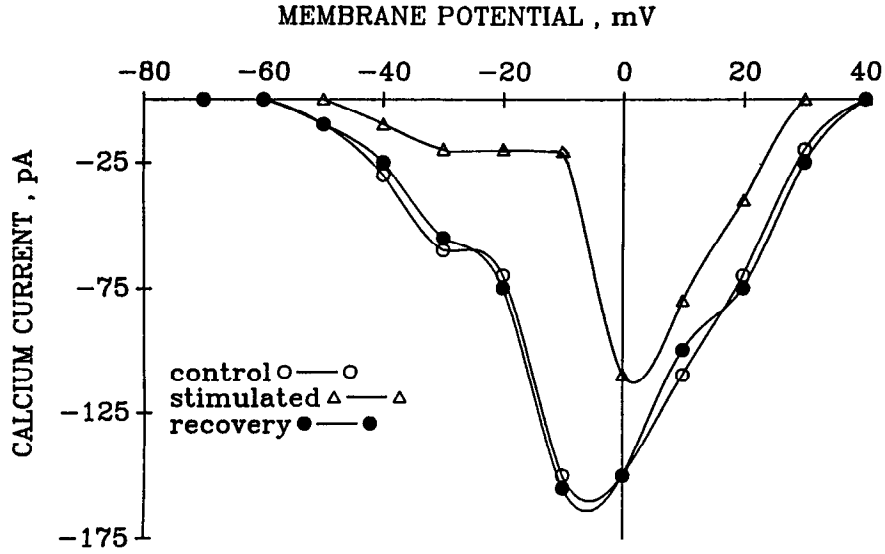

Figure 3. Stalk stimulation reversibly reduces inward calcium currents evoked from −90 mV holding potential. Current voltage traces from a typical experiment show Ca^{2+} currents (pA) before (*open circles*) and after stimulation of the pituitary stalk (*open triangles*) and following recovery (*closed circles*). In these experiments, holding potential was at −90 mV, and voltage was stepped in 10 mV increments to the levels indicated by the data points. Voltage steps were elicited at 3–5 sec intervals to prevent possible time-dependent inactivation of the currents.

cells and a proportion of them displayed spontaneous activity. When cells were impaled with electrodes to isolate Ca^{2+} responses as described above, Ca^{2+} spikes could be elicited by intracellular injection of depolarizing current. When these Ca^{2+}-based action potentials were elicited at 2 sec intervals, stimulation of the pituitary stalk (5–10 V, 1 msec duration, 1–10 pulses at 50 Hz) abolished the Ca^{2+} spike for periods of several seconds ($n = 6$, Fig. 1). Intracellular injection of hyperpolarizing current pulses indicated that there was no change of membrane input resistance at a time when the Ca^{2+} spikes were depressed. The absence of a hyperpolarization or a change in input resistance in response to stalk stimulation confirms that the combination of pharmacological blockers used has abolished the DA-activated K^+ conductance known to be present in the melanotroph (Williams et al., 1989a). Only Ca^{2+} spikes elicited with current pulses slightly suprathreshold were abolished by stalk stimulation. Ca^{2+} spikes evoked with voltage pulses much greater than threshold for the spike were usually not abolished; rather, the duration of the spike was reversibly decreased ($n = 3$). To more rigorously characterize this effect, single-electrode voltage-clamp technique was used to analyze the Ca^{2+} currents. In all cases, these currents were subsequently shown to be blocked by perfusion of cobalt or cadmium, and values reported for Ca^{2+} currents have been subtracted for the leak currents obtained in the presence of cobalt. Ca^{2+} currents corresponding to the T, N, and L types (Nowycky et al., 1985) and previously described by us (Williams et al., 1990) were obtained (Fig. 2). When the holding potential was −90 mV, stimulation of the pituitary stalk inhibited both the low- and the high-threshold peak inward Ca^{2+} current. Currents elicited by 200 msec pulses from −90 to 0

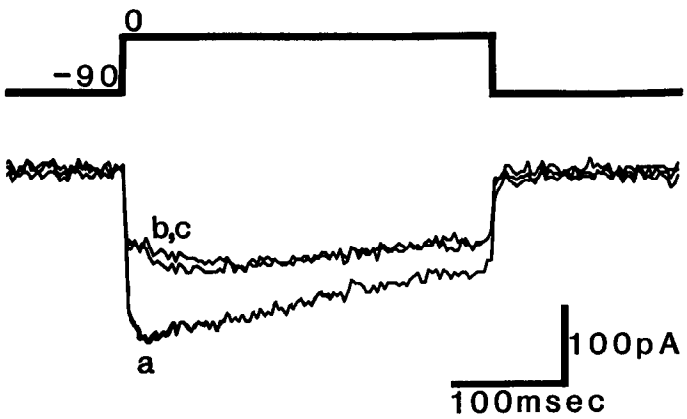

Figure 4. Stalk stimulation abolishes a transient Ca^{2+} current. The Ca^{2+} current elicited by a 400 msec voltage pulse from a holding potential of −90 mV to a test potential of 0 mV (*a*) had an inactivating N component. When the same pulse was elicited 150 msec after a prepulse (200 msec duration) to a potential of +10 mV the initial, more rapidly decaying current was abolished while a sustained, nondecaying current persisted (*b*). A similar response was observed when the test pulse was preceded by a stimulus to the pituitary stalk (*c*). A single pulse to the stalk (10 V, 1 msec) abolished the same transient current that was eliminated by the prepulse.

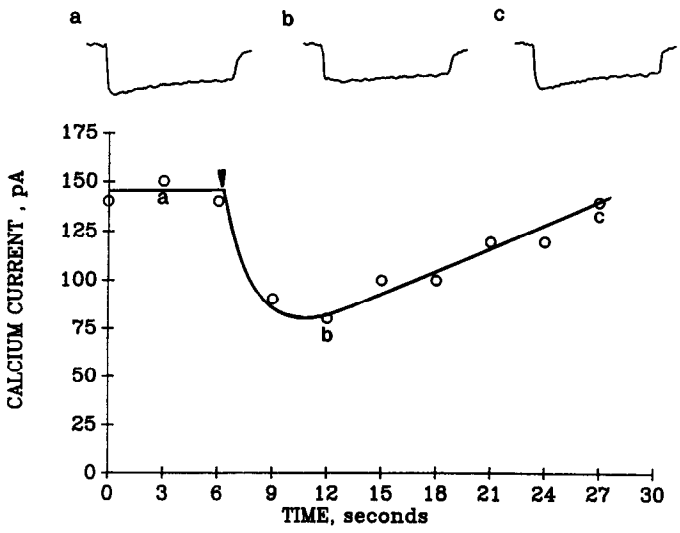

Figure 5. Duration of depressant action of stalk stimulation on Ca^{2+} currents. *Top,* Calcium currents elicited in response to 200 msec voltage steps to 0 mV from a holding potential of −90 mV before (*a*), and 6 sec (*b*) and 22 sec (*c*) after stimulation of the pituitary stalk. *Lower,* Peak Ca^{2+} currents and the time course of their reduction following stimulation of the pituitary stalk (1 pulse, 10 V, 1 msec) at the time indicated by the *arrow.* The letters *a–c* indicate the points at which the current traces in the upper part were taken.

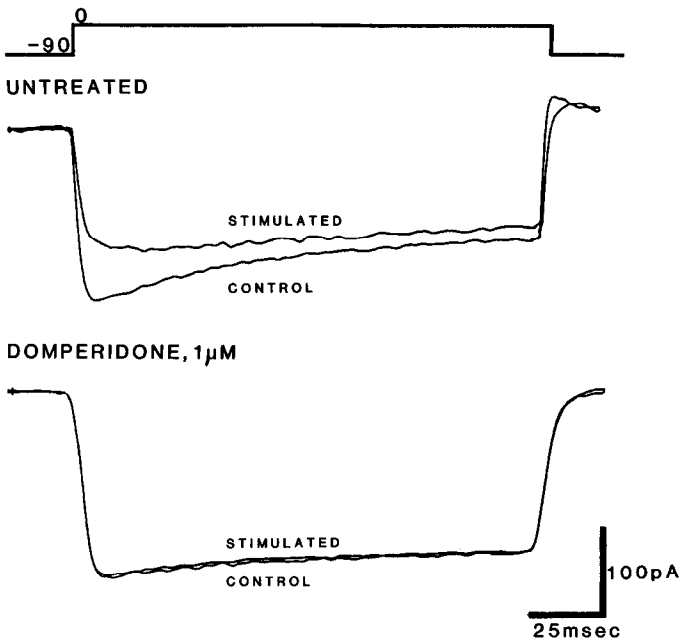

Figure 6. Domperidone, a D-2 receptor antagonist, blocks synaptic inhibition of Ca^{2+} currents. Current traces were taken from cells held at −90 mV and stepped up to 0 mV. *Control* and *Stimulated* refer to traces taken immediately before and 5 sec after stimulation of the pituitary stalk. Before addition of domperidone, stalk stimulation clearly reduced Ca^{2+} currents (*upper*). Following addition of the antagonist (*lower*), this effect was abolished.

mV were reduced to 62 ± 12% (mean ± SD, $n = 8$) of control values, while currents elicited by pulses from −30 to 0 mV were 96 ± 6% of control (Fig. 2). The inhibition of the Ca^{2+} currents was reversible (Fig. 3) and relatively consistent from cell to cell. Examination of the waveforms of the currents after stalk stimulation suggested that the noninactivating current persisted while the transient current was inhibited. This pattern of inhibition of the Ca^{2+} current was consistent with the preferential inhibition of the N and T Ca^{2+} currents with an apparent sparing of the L type current.

In our previous paper (Williams et al., 1990), we had described how prepulses to positive potentials appeared to inhibit the N type Ca^{2+} current. We therefore compared the effects of stalk stimulation and a 200 msec prepulse to a potential of +10 mV on the cell's response. As seen in Figure 4, both the prepulse and stalk stimulation produce a similar inhibition of the peak current, providing further evidence that stalk stimulation is selectively abolishing transient Ca^{2+} currents. The duration of the inhibition of the peak Ca^{2+} currents by stimulation of the pituitary stalk (Fig. 5) was similar to the previously observed duration of Ca^{2+} spike inhibition (Fig. 1).

As the presence of bicuculline in the perfusate would block the inhibitory actions of GABA at the $GABA_A$ receptor on these cells (Williams et al., 1989b), DA is the probable transmitter mediating these inhibitory responses to stalk stimulation (Williams et al., 1989a). We therefore examined the potential role of DA in the inhibition of Ca^{2+} currents by perfusing the D-2 antagonist, domperidone (1 μM; $n = 3$) over the preparation. Domperidone totally blocked the inhibitory effects of stalk stimulation on the Ca^{2+} currents (Figs. 6, 7) without having any effect on leak current. We next determined if the inhibition of Ca^{2+} currents elicited by stalk stimulation could be mimicked by quinpirole (5 μM), an agonist at the D-2 receptor. Quinpirole mimicked the action of pituitary stalk stimulation by inhibiting Ca^{2+} currents evoked from a holding potential of −90 mV, but not −30 mV (Fig. 8; $n = 5$).

Some of dopamine's actions on hormone release from the melanotroph have been shown to be mediated by G-proteins (Cote et al., 1985). Therefore, experiments were carried out to assess the role of G-proteins in the inhibition caused by synaptically released DA or exogenously applied DA agonists. In rats pretreated with pertussis toxin, which inactivates some G-proteins, only 3 of 12 cells showed any inhibition of Ca^{2+} currents due to stalk stimulation. This is significantly different ($p < 0.05$; χ^2 test) than the 100% of cells ($n = 30$) which normally display inhibition of Ca^{2+} currents due to stalk stimulation. Quinpirole was also without effect on Ca^{2+} currents in melanotrophs taken from pertussis toxin-treated rats ($n = 3$; Fig. 9).

In a second series of experiments, melanotrophs were impaled with electrodes containing GTPγS (50 μM), a GTP analog which

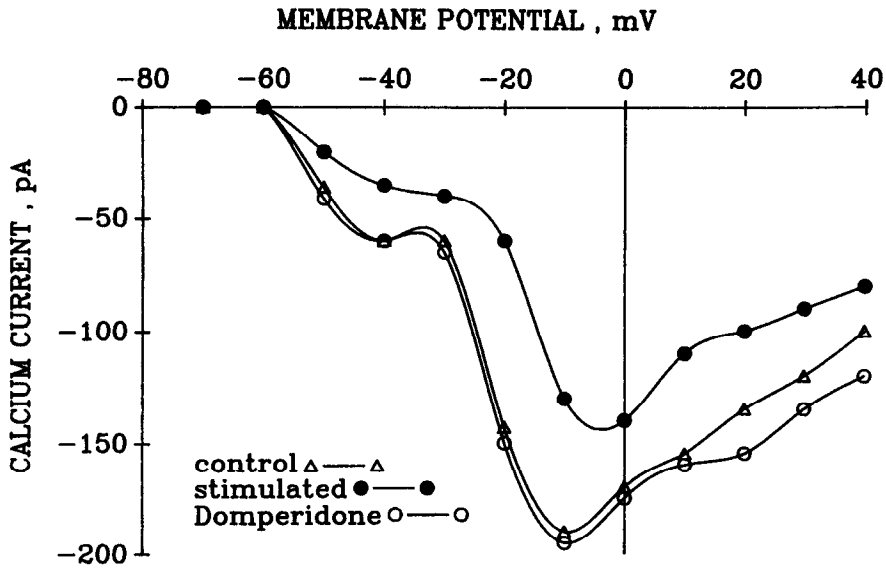

Figure 7. Domperidone, a D-2 antagonist, blocks the inhibitory effects of stalk stimulation on Ca^{2+} currents. Current voltage traces from a cell at a holding potential of −90 mV and stepped in 10 mV increments to membrane potentials indicated by the data points. Control peak Ca^{2+} currents were obtained (*open triangles*) after which Ca^{2+} currents were again elicited following stalk stimulation in the absence of (*closed circles*) and the presence of the D-2 antagonist domperidone (1 μM, *open circles*).

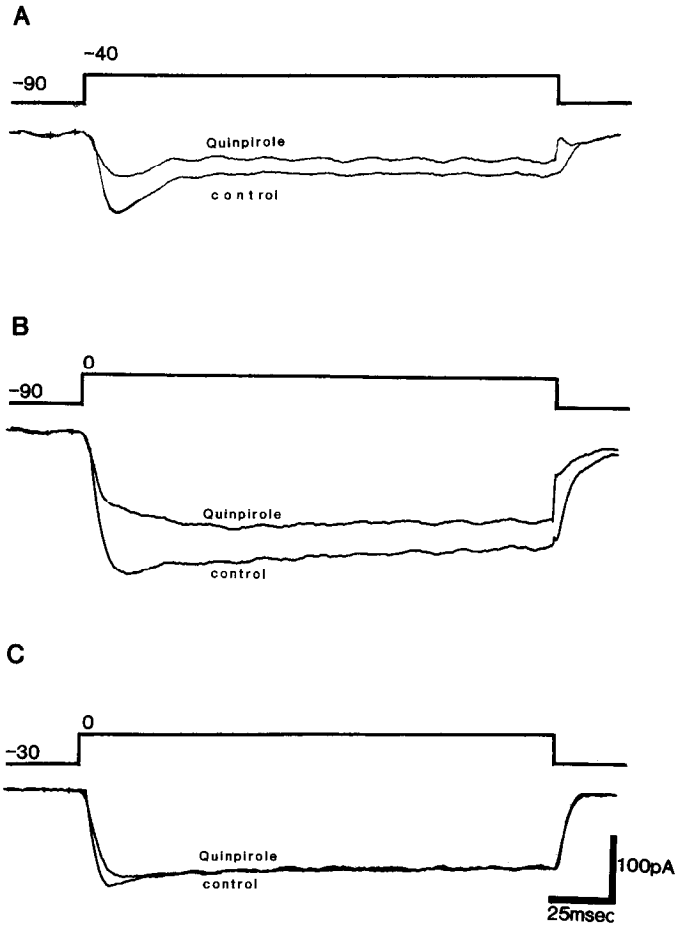

Figure 8. Quinpirole, a D-2 agonist, inhibits inactivating, but not noninactivating, inward Ca^{2+} currents. From holding potentials indicated to the *left* of each voltage trace, the voltage was stepped up to the values indicated above each voltage trace. Addition of quinpirole (5 μM) inhibited both the low- (*A*) and the high-threshold (*B*) Ca^{2+} currents but did not appreciably inhibit the noninactivating current (*C*) elicited from a holding potential of −30 mV.

irreversibly activates G-proteins. In all cells recorded with GTPγS in the electrode ($n = 15$), Ca^{2+} current amplitude was lower than in cells recorded without GTPγS, and neither stalk stimulation nor quinpirole caused a further reduction in current amplitude. Inspection of current records revealed no inactivating Ca^{2+} current, suggesting that the T- and N-type currents were abolished. To confirm this, voltage pulses were applied from a holding potential of −90 mV, which would activate T, N, and L types, or from a holding potential of −30 mV, which would activate only L type. In control cells, a significant difference was seen in response to these 2 protocols (Williams et al., 1990), reflecting inactivation of N-type current at the −30 mV holding potential. In cells impaled with electrodes containing GTPγS, however, the response from −90 mV was virtually the same as the response from −30 mV (Fig. 10), suggesting that the N type current was abolished in these cells. Comparison of individual current responses to a 200 msec test pulse from −90 to 0 mV also suggests that the initial rapidly inactivating current is abolished in GTPγS-treated cells.

Discussion

Ca^{2+} currents similar to the T, N, and L subtypes have been identified in pituitary melanotrophs (Taleb et al., 1986; Williams et al., 1990). As we described in our previous paper (Williams et al., 1990), there are reports of Ca^{2+} currents that do not fall readily into the classification scheme proposed by Nowycky et al. (1985); however, we believe that the currents we describe here are sufficiently similar that the T, N, and L designations are appropriate and useful. In the present study, we have shown that both synaptically released DA and an exogenously applied D-2 agonist reduce inactivating (T and N) Ca^{2+} currents while leaving the noninactivating (L) type unaffected. Our conclusions are based both on the fact that currents activated from a holding potential of −30 mV are unaffected by stalk stimulation or D-2 agonists and from an examination of the waveforms of the Ca^{2+} currents before and after dopaminergic activation. In both cases, noninactivating currents (L type) persist and inactivating (T and N) are reduced. As we have previously demonstrated that DA

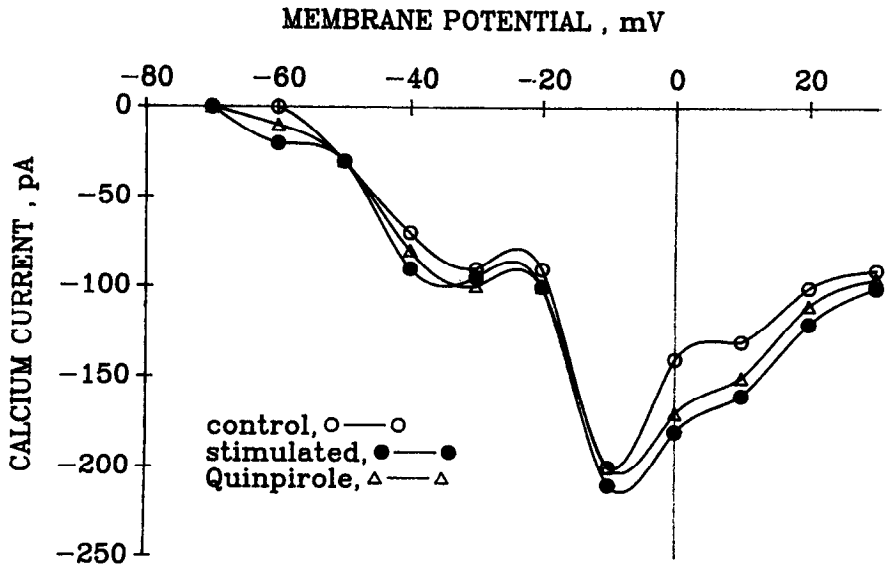

Figure 9. Pertussis toxin pretreatment blocks dopamine-mediated inhibition of Ca^{2+} currents. Current voltage plots show peak Ca^{2+} currents obtained in response to 200 msec test pulses to levels indicated by the data points from a holding potential of −90 mV. *Open circles* are control responses, *closed circles* are responses following stalk stimulation, and *open triangles* are Ca^{2+} currents in the presence of quinpirole. It can be seen that stalk stimulation and quinpirole were without effect on the Ca^{2+} currents in these cells.

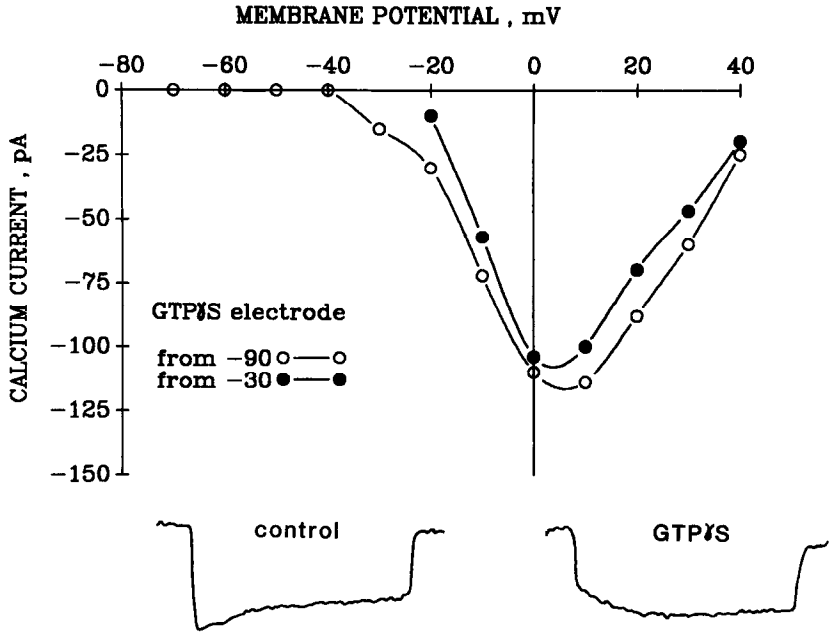

Figure 10. GTPγS inhibits T- and N-type Ca^{2+} currents. Currents recorded in response to voltage pulses from a holding potential of −90 or −30 mV to test voltages between −70 and +40 mV. Plot shows essentially similar current response from the 2 different holding potentials. *Inset,* Leak-subtracted currents from a control cell and from a cell impaled with an electrode containing GTPγS. Currents are in response to 200 msec voltage pulse from −90 to 0 mV.

activates a K^+ conductance in these cells (Williams et al., 1989a), it is important to determine that the effect on Ca^{2+} currents is not secondary to a change in K^+ conductance. In our experiments, in order to isolate the Ca^{2+} current, it was necessary to utilize a number of K^+ channel blockers; we have previously shown that a combination of intracellular injection of Cs^+ and TEA plus extracellular 4-AP blocks all voltage-activated K^+ conductances (Williams et al., 1990). We also have observed previously (Williams et al., 1989a) that the DA IPSP due to an increased K^+ conductance was blocked when intracellular electrodes contained Cs^+ and TEA. Also, no change in the holding currents due to activation of an outward current was seen in the present experiments following stalk stimulation. Finally, it would appear unlikely that a shunting of the membrane due to a DA-activated K^+ conductance, if present, would affect only the T and N currents while leaving the L current intact.

Our demonstration that DA agonist application reduces Ca^{2+} spikes is consistent with the previous observations of Douglas and Taraskevich (1978) that DA slows action potential frequency in pars intermedia cells. In a subsequent study of the effects of DA on Ca^{2+} spikes in these cells (Douglas and Taraskevich, 1982), DA was shown to inhibit Ca^{2+} spikes, but this effect was seen in only 3 of 11 cells whereas in our experiments stalk stimulation or DA agonist application was always found to inhibit Ca^{2+} currents. The reasons for this discrepancy are possibly related to the high (10 mM) levels of Ca^{2+} used in the previous study or to the possible metabolism or oxidation of DA before it reached its site of action.

Our observation of the dopaminergic reduction of Ca^{2+} currents is in keeping with many recent observations on transmitter modulation of Ca^{2+} currents (see Dunlap and Fischbach, 1981; Miller, 1987; Tsien et al., 1988). In particular, they add to the growing evidence that DA depresses Ca^{2+} currents in a variety of cells (Lewis et al., 1984; Paupardin-Tritsch et al., 1985; Israel et al., 1987; Malgaroli et al., 1987; Harris-Warrick et al., 1988). To the best of our knowledge, this is the first demonstration of *synaptic* modulation by DA of a Ca^{2+} current in the mammalian nervous system. Evidence for this is based on 2 observations: (1) the similarity between the action of the D-2 agonist quinpirole and that of synaptic stimulation on the Ca^{2+} current and (2) the ability of a specific D-2 antagonist domperidone (Baudry et al., 1979; Laduron and Leysen, 1979; Lazareno and Nahorski, 1982) to block the inhibitory effect of stalk stimulation on Ca^{2+} currents.

There is now considerable evidence that certain voltage-dependent Ca^{2+} channels are controlled by G-proteins (Holtz et al., 1986; reviewed in Rosenthal et al., 1988). Indeed, in Helix neurons there is evidence that an α_{40} subunit of a GTP-binding protein immunologically related to G_o mediates a DA-induced decrease in Ca^{2+} currents (Harris-Warrick et al., 1988). The inhibition of Ca^{2+} currents in the melanotroph also appears to be G-protein mediated since (1) pertussis toxin, which catalyzes the ADP-ribosylation of G_i and G_o, thus rendering them inactive, blocks the effects of DA and (2) the effects of DA are mimicked by GTPγS, which irreversibly activates G-proteins.

It is interesting that we have previously observed that the DA-induced activation of a K^+ current in the melanotroph is also mediated by G-proteins (Williams et al., 1989a). There is also evidence that DA receptor activation reduces adenylate cyclase activity in these cells (reviewed in Cote et al., 1985) and that this event is also G-protein mediated (Cote et al., 1984). The interrelationship between these different systems remains to be elucidated.

Our results underline the utility of melanotroph as an ideal preparation for investigation of the electrophysiological and biochemical consequences of D-2 receptor activation. In particular, the compact nature of the cell circumvents difficulties which have arisen in other systems (see Lewis et al., 1984), wherein it has been difficult to examine DA-induced currents because their dendritic locus resulted in potential space-clamp problems. In addition, the lack of interneurons and complicated circuitry in the intermediate lobe greatly simplifies the interpretation of synaptically mediated events. The melanotroph, therefore, represents a powerful model system for the study of dopaminergic neurotransmission and the neuronal control of hormone release.

References

Aghajanian, G. K., and Y. Y. Wang (1986) Pertussis toxin blocks the outward currents evoked by opiate and α_2 agonists in locus coeruleus neurons. Brain Res. *371:* 390–394.

Baudry, M., M. P. Martres, and J. C. Schwartz (1979) ^{3}H-domperidone: A selective ligand for dopamine receptors. Arch. Pharmacol. *308:* 231–237.

Baumgarten, H. G., A. Bjorklund, A. F. Holstein, and A. Nobin (1972) Organization and ultrastructural identification of catecholamine nerve terminals in the neural lobe and pars intermedia of the rat pituitary. Z. Zellforsch. *126:* 483–517.

Colmers, W. F., and Q. J. Pittman (1989) Presynaptic inhibition by neuropeptide Y and baclofen in hippocampus: Insensitivity to pertussis toxin treatment. Brain Res. *498:* 99–104.

Cote, T. E., E. A. Frey, and R. D. Sekura (1984) Altered activity of the inhibitory guanyl nucleotide-binding component (N_i) induced by pertussis toxin. J. Biol. Chem. *259:* 8693–8698.

Cote, T. E., E. A. Frey, R. D. Sekura, and J. W. Kebabian (1985) Dual regulation of adenylate cyclase activity and hormone release in the intermediate lobe of the rat pituitary gland: Evidence for the involvement of membrane components of the stimulatory β_2-adrenergic system and of the inhibitory D-2 dopaminergic system. Adv. Cyclic Nucleotide Res. *19:* 151–167.

Douglas, W. W., and P. S. Taraskevich (1978) Action potentials in gland cells of rat pituitary pars intermedia: inhibition by dopamine, an inhibitor of MSH secretion. J. Physiol. (Lond.) *285:* 171–184.

Douglas, W. W., and P. S. Taraskevich (1980) Calcium component to action potentials in rat pars intermedia cells. J. Physiol. (Lond.) *309:* 623–630.

Douglas, W. W., and P. S. Taraskevich (1982) Slowing effects of dopamine and calcium-channel blockers on frequency of sodium spikes in rat pars intermedia cells. J. Physiol. (Lond.) *326:* 201–211.

Dunlap, K., and G. D. Fischbach (1981) Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. J. Physiol. (Lond.) *317:* 519–535.

Goldman, M. E., M. Beaulieu, J. W. Kebabian, and R. L. Eskay (1983) α-Melanocyte stimulating hormone-like peptides in the intermediate lobe of the rat pituitary gland: Characterization of content and release *in vitro.* Endocrinology *112:* 435–441.

Harris-Warrick, R. M., C. Hammond, D. Paupardin-Tritsch, V. Homburger, B. Rouot, J. Bockaert, and H. M. Gerschenfeld (1988) An α_{40} subunit of a GTP-binding protein immunologically related to G_o mediates a dopamine-induced decrease of Ca^{2+} current in snail neurons. Neuron *1:* 27–32.

Holtz, G. G. IV, S. G. Rane, and K. Dunlap (1986) GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels. Nature *319:* 670–672.

Howe, A. (1973) The mammalian pars intermedia: A review of its structure and function. J. Endocrinol. *59:* 385–409.

Israel, J. M., C. Kirk, and J. D. Vincent (1987) Electrophysiological responses to dopamine of rat hypophysial cells in lactotroph-enriched primary cultures. J. Physiol. (Lond.) *390:* 1–22.

Laduron, P. M., and J. E. Leysen (1979) Domperidone, a specific *in vitro* dopamine antagonist, devoid of *in vivo* central dopaminergic activity. Biochem. Pharmacol. *28:* 2161–2165.

Lazareno, S., and S. R. Nahorski (1982) Selective labelling of dopamine (D_2) receptors in rat striatum by [^{3}H] domperidone but not by [^{3}H] Spiperone. Eur. J. Pharmacol. *81:* 273–285.

Lewis, D. V., G. B. Evans, and W. A. Wilson (1984) Dopamine reduces slow outward current and calcium influx in burst-firing neuron R15 of *Aplysia.* J. Neurosci. *4:* 3014–3020.

MacVicar, B. A., and Q. J. Pittman (1986) Novel synaptic responses mediated by dopamine and γ-aminobutyric acid in neuroendocrine cells of the intermediate pituitary. Neurosci. Lett. *64:* 35–40.

Malgaroli, A., L. Vallar, F. R. Elahi, T. Pozzan, A. Spada, and J. Meldolesi (1987) Dopamine inhibits cytosolic Ca^{2+} increases in rat lactotroph cells: Evidence of a dual mechanism of action. J. Biol. Chem. *262:* 13920–13927.

McCleskey, E. W., A. P. Fox, D. Feldman, and R. W. Tsien (1986) Different types of calcium channels. J. Exp. Biol. *124:* 177–190.

Miller, R. J. (1987) Multiple calcium channels and neuronal function. Science *238:* 46–52.

Nowycky, M. C., A. P. Fox, and R. W. Tsien (1985) Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature *316:* 440–443.

Paupardin-Tritsch, D., L. Colombaioni, P. Deterre, and H. M. Gerschenfeld (1985) Two different mechanisms of calcium spike modulation by dopamine. J. Neurosci. *5:* 2522–2532.

Pittman, Q. J., J. Disturnal, C. Riphagen, W. L. Veale, and L. Bauce (1985) Perfusion techniques for neural tissue. In *Neuromethods, Vol. 1: General Neurochemical Techniques,* A. A. Boulton and G. B. Baker, eds., pp. 279–303, Humana Press, Inc.,

Rosenthal, W., J. Hescheler, W. Trautwein, and G. Schultz (1988) Control of voltage-dependent Ca^{2+} channels by G protein-coupled receptors. FASEB J. *2:* 2784–2790.

Taleb, O., J. Trouslard, B. A. Demeneix, and P. Fletz (1986) Characterization of calcium and sodium currents in porcine pars intermedia cells. Neurosci. Lett. *66:* 55–60.

Taraskevich, P. S., S. A. Tomiko, and W. W. Douglas (1986) Electrical stimulation of neurointermediate lobes of mice elicits calcium-dependent output of melanocyte-stimulating hormone. Brain Res. *379:* 390–393.

Tilders, F. J. H., A. H. Mulder, and P. G. Smelik (1975) On the presence of a MSH-release inhibiting system in the rat neurointermediate lobe. Neuroendocrinology *19:* 125–130.

Tsien, R. W., D. Lipscombe, D. V. Madison, K. R. Bley, and A. P. Fox (1988) Multiple types of neuronal calcium channels and their selective modulation. Trends Neurosci. *11:* 431–438.

Williams, P. J., B. A. MacVicar, and Q. J. Pittman (1989a) A dopaminergic IPSP mediated by an increased potassium conductance. Neuroscience *31:* 673–681.

Williams, P. J., B. A. MacVicar, and Q. J. Pittman (1989b) Identification of a GABA-activated chloride mediated synaptic potential in rat pars intermedia. Brain Res. *483:* 130–134.

Williams, P. J., B. A. MacVicar, and Q. J. Pittman (1990) Electrophysiological properties of neuroendocrine cells of the intact rat pars intermedia: Multiple calcium currents. J. Neurosci. *10:* 748–756.