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DOPAMINE ACTIONS ON CALCIUM CURRENTS, POTASSIUM CURRENTS AND HORMONE RELEASE IN RAT MELANOTROPHS

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SUMMARY

1. Intracellular and whole-cell recordings were made from primary cultures of rat intermediate pituitary cells; β-endorphin secretion was also measured by radioimmunoassay. The effects of dopamine receptor activation on hormone secretion, calcium currents and resting potassium conductance were compared.

2. Spontaneous sodium-dependent action potentials occurred in 82% of cells recorded with intracellular microelectrodes and 64% of cells recorded with whole-cell patch electrodes; the same proportion of cells showed spontaneous calcium-dependent depolarizations in the presence of tetrodotoxin.

3. Calcium currents recorded from holding potentials of -90 or -70 mV showed transient and sustained components, both of which activated at -40 mV and had similar current–voltage relations. Bay K 8644 (1 μM) increased both components by about 130% while nifedipine (1–10 μM) decreased them by a maximum of 30%. Nickel (500 μM) inhibited transient and sustained components by 68 and 50%; cadmium (100 μM) abolished the current. ω-Conotoxin (1 μM) reversibly inhibited the transient component by 26%.

4. The dopamine D_2 receptor agonist, quinpirole (0·1–10 μM) inhibited transient and sustained components in all cells by a maximum of 40 and 25% respectively. Quinpirole did not alter the time course of the current.

5. Quinpirole (1–100 nM) hyperpolarized 90% of cells from which intracellular recordings were made and 55% of cells recorded from with whole-cell patch pipettes. Maximum hyperpolarization of 16 ± 4 mV from a resting potential of -44 ± 5 mV was observed with 100 nM-quinpirole; concentration producing half-maximal effect was 3 nM. The hyperpolarization resulted from an increase in potassium conductance.

6. Quinpirole (1–100 nM) decreased basal β-endorphin secretion by 55% and abolished secretion stimulated by Bay K 8644 or isoprenaline; concentrations producing half-maximal inhibitions were 5–10 nM. Tetrodotoxin (1 μM), nifedipine (1 μM), nickel (500 μM) and cadmium (100 μM) did not alter basal or stimulated secretion although higher concentrations of cadmium did inhibit stimulated hormone release.

7. Pertussis toxin pre-treatment prevented all actions of quinpirole.

8. Thus, concentrations of quinpirole that abolished stimulated hormone secretion did not alter calcium currents; conversely, concentrations of calcium channel

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blockers that partially or completely inhibited calcium currents did not alter basal or stimulated secretion. These results may indicate that calcium influx through the voltage-dependent calcium channels measured in these experiments does not contribute significantly to hormone release from melanotrophs.

9. The same concentrations of quinpirole activated a potassium conductance and inhibited hormone release. It is concluded that membrane hyperpolarization may be the primary mechanism by which dopamine receptor activation inhibits hormone release from melanotrophs.

INTRODUCTION

The dopamine D_2 receptor is one of several G-protein-coupled receptors whose activation is known to inhibit neurotransmitter or hormone release from neurones and endocrine cells (North, 1989). This dopamine receptor is also known to couple to activation of potassium currents and to inhibition of calcium currents in many neuronal and endocrine tissues (see reviews by Lacey, 1989; Nicoll, Malenka & Kauer, 1990) and either, or both, of these actions have been suggested as the mechanism by which hormone or transmitter release is inhibited. One way to approach this question is to compare directly the actions of dopamine D_2 receptor agonists on these three effects in the same tissue under the same conditions. Such an approach is unsatisfactory in neuronal tissue because alterations in ionic currents are recorded in neuronal soma in response to activation of somal receptors, while inhibition of transmitter release occurs at a physically remote site (i.e. the nerve terminal) in response to activation of nerve terminal receptors. This complication does not apply to electrically excitable endocrine cells where ionic currents can be recorded at the same macroscopic site (the cell body) as hormone release occurs.

Considerable information exists concerning dopamine actions in pituitary melanotrophs, which synthesize and post-translationally process a prohormone, pro-opiomelanocortin (POMC), into peptides for release. The characteristic hormones secreted by melanotrophs are α-melanocyte-stimulating hormone (αMSH) and acetylated β-endorphin, which are stored in and secreted from secretory granules on an equimolar basis (Mains & Eipper, 1979; Eipper & Maine, 1980). Dopamine D_2 receptor activation inhibits basal and stimulated αMSH and β-endorphin release through a cyclic AMP-independent mechanism (Munemura, Eskay & Kebabian, 1980; Miyazaki, Goldman & Kebabian, 1984; Beaulieu, Felder & Kebabian, 1986); it also activates a potassium conductance and inhibits a calcium current in a pertussis-toxin-sensitive manner (Williams, MacVicar & Pittman, 1989, 1990; Cota & Hiriart, 1989). Membrane hyperpolarization resulting from an increased potassium conductance and/or direct inhibition of a calcium current are especially attractive hypotheses to explain inhibition of hormone release in melanotrophs because these cells exhibit spontaneous calcium-dependent membrane depolarizations (Douglas & Taraskevich, 1985).

In the present study, we used intracellular and whole-cell recordings to measure membrane hyperpolarization or outward potassium current in response to dopamine receptor activation, whole-cell recordings to isolate and measure calcium currents, and radioimmunoassays to measure β-endorphin secretion. The primary aim was to directly compare dopamine actions on hormone release, calcium currents and

potassium currents. Few such correlative studies have been reported but these types of experiments, in which other cellular responses are measured under conditions where hormone release occurs, are important now that it is clear that activation of a single G-protein-linked receptor may couple to many effector responses in the same cell. For example, the vast majority of electrophysiological recordings of calcium currents have been carried out at temperatures (usually room temperature) which are some 10–12 °C lower than required to support stimulated hormone release from mammalian corticotrophs measured by radioimmunoassays (Stack, 1990) and by membrane capacitance recordings (Thomas, Surprenant & Almers, 1990). Preliminary reports of portions of this work have been presented (Stack, Surprenant & Allen, 1987; Stack, Zlatnick, Allen & Surprenant, 1990).

METHODS

Cell preparation

Neurointermediate lobes (NIL) were dissected from pituitaries from male Sprague–Dawley rats, killed by halothane inhalation (200–220 g, Banton and Kingman), and collected in HEPES buffer, which contained (mM): NaCl, 137; KCl, 5; Na_2HPO_4, 0·7; glucose, 10; HEPES, 25; adjusted to pH 7·4 with NaOH. This was also used for enzyme solutions. The lobes were quartered and the tissue fragments were collected and pelleted by centrifugation (500 r.p.m. for 5 min). The supernatant was replaced with collagenase buffer (0·5 ml/NIL), which was 0·2% collagenase type V and 0·4% bovine serum albumin (BSA; both from Sigma), and the tissue fragments were resuspended and incubated for 30 min at 37 °C. Tissue fragments were triturated and further incubated for 15 min in trypsin buffer at 0·5 ml/NIL, which was composed of 0·05% trypsin (Sigma) and 0·4% BSA added directly to the collagenase buffer. The tissue fragments were completely dispersed using a small bore pasteur pipette. Cells were collected by centrifugation (500 r.p.m. for 5 min) and the cell pellet was resuspended in Dulbecco's modified Eagle's medium (DMEM) without calcium (Gibco, formula no. 81-5210) and supplemented with 0·05% BSA. The cell suspension was plated onto 12 mm diameter glass cover-slips (Belco) in 25 μl aliquots containing 12·5–25% NIL for electrophysiology experiments, or into 96-well tissue culture plates (Nunc) in 25 μl aliquots containing 30% NIL for secretion experiments. DMEM (Gibco, no. 430-1600) supplemented with 10% heat-inactivated horse serum (Gibco) was added to the cells 1 h after plating. Cells were cultured for 5–7 days. For pertussis toxin experiments, cells were pretreated with 100 ng/ml pertussis toxin for 16 h immediately prior to experiments.

Hormone secretion studies

Cells were washed five times in a 90 min period, the subsequent 2 h was divided into four periods of 30 min. After each period the medium was removed and saved, and replaced with DMEM or DMEM plus drug with a volume of 50 μl. The samples were centrifuged to remove any debris and 30 μl of the supernatant was assayed for β-endorphin immunoactivity as described by Rees, Cook, Kendall, Allen, Kramer, Ratcliffe & Knight (1971) and modified by Allen, Herbert, Hinman, Shibuya & Pert (1978) using the antiserum (Sugar) which is specific for the midportion region of the β-endorphin molecule and cross-reacts with all forms of β-endorphin as well as β-lipotrophin and POMC (Hatfield, Allen, Stack & Ronnekleiv, 1988). Secretion rates were normalized using a ratio of the β-endorphin immunoactivity secreted during drug treatment to the β-endorphin immunoactivity secreted during basal conditions and data are expressed as a percentage of control.

Electrophysiological methods

Intracellular recordings. Conventional intracellular recordings were obtained using an Axoclamp-2A amplifier; microelectrodes were filled with 1 M-KCl and had resistances of 60–100 MΩ. Physiological saline was of the following composition (mM): NaCl, 126; NaH_2PO_4, 1·2; $MgCl_2$, 1·2; $CaCl_2$, 2·5 or 5; KCl, 5; $NaHCO_3$, 25; glucose, 11; gassed with 95% O_2 and 5% CO_2 flowed at 5 ml/min through the bath (volume 0·7 ml). Temperature was maintained at 34–36 °C.

Whole-cell recordings. Tight-seal (5–10 GΩ), whole-cell recordings were obtained using an

Axopatch 1B amplifier and patch pipettes with resistances of 4–6 MΩ; cell input resistance measured with potassium gluconate-based internal solution was 5–20 GΩ and no series compensation was necessary. Membrane potentials reported are corrected for liquid junction potentials when caesium or potassium (11 and 9 mV negative) gluconate-based internal solutions were used. Calcium currents were isolated using an internal solution of (mM): caesium glutamate or caesium gluconate, 160; EGTA, 10; HEPES, 5; Mg-ATP, 4·5; GTP, 0·1; and adjusted to pH 7·4 with CsOH, and an external solution of (mM): NaCl, 166; CsCl, 3; $CaCl_2$, 2·5 or 5; glucose, 10; HEPES, 5; tetrodotoxin (TTX), 0·001 and adjusted to pH 7·4 with NaOH. After it was determined that 100 μM-cadmium was sufficient to abolish all time- and voltage-dependent currents (e.g. Fig. 3), all calcium currents were measured after subtraction of the current which remained in the presence of 300 μM-cadmium. In all other whole-cell recordings potassium gluconate or potassium glutamate was used in place of caesium gluconate in the internal solution and the external solution contained (mM): NaCl, 160; KCl, 5; $MgCl_2$, 1; $CaCl_2$ 2·5 or 5; HEPES, 10 and glucose, 11. Drugs were applied by a six-barrelled device that allowed for rapid solution changes similar to that described by Johnson & Ascher (1987), modified so that all solutions were delivered to the bath at 34–37 °C. The tubing carrying the solutions traversed a heated water jacket prior to entry into the recording chamber. Flow was regulated by gravity and the flow rate was kept constant to maintain constant temperature between barrels.

Data analysis

Because the major aim of this study was to make comparisons between dopamine actions on ionic currents and hormone secretion, several precautions were undertaken toward this goal: preparations used for secretion and electrophysiological experiments were identical; they were dispersed concomitantly and, except that lower density platings were used for electrophysiological recordings, were used at the same time in culture (5–7 days after dispersal). Cover-slips used for secretion and electrophysiological studies were taken at random intervals over the course of this study and subjected to β-endorphin immunohistochemistry (Hatfield *et al.* 1988); in all cases (n = 8) over 95% of cells were immunopositive. In electrophysiological experiments each cell tested with any one agonist or antagonist was obtained from a separate set of dispersals. Measurements were made only on those cells in which the response returned to within 5% of control after drug wash-out. All results are expressed as mean ± S.E.M.; tests of significance were by one-way analysis of variance after *ad hoc* Student's t test. Differences were considered significant at $P < 0·01$ for all data.

Drugs used were quinpirole (dopamine D_2 receptor agonist, Lilly), dopamine, isoprenaline, forskolin, tetrodotoxin and guanosine 5′-*O*-(3-thiotriphosphate) (GTP-γ-S; Sigma), pertussis toxin (List), ω-conotoxin (RBI), sulpiride (dopamine receptor antagonist, Ravizza), and Bay K 8644 (Calbiochem).

RESULTS

The overall purpose of these experiments was to determine concentration–response relations for the actions of calcium channel blockers and dopamine receptor agonists on three distinct cellular responses, to test the hypothesis that dopamine inhibits hormone release in melanotrophs by activating a potassium conductance and/or by inhibiting a calcium current. Thus, results of experiments concerning potassium conductance and resting membrane properties are first presented. Secondly, similar experiments in which calcium currents were examined are described. Finally, results of parallel hormone-release experiments and their correlation with the former two sets of studies are presented.

Spontaneous action potential activity

Figure 1 illustrates typical spontaneous action potential activity recorded from melanotrophs with intracellular microelectrodes; thirty-five of forty-three (82%) cells exhibited spontaneous activity with the frequency of the TTX-sensitive action

potentials averaging 1·5 ± 0·2 Hz (Fig. 1). Irregular membrane potential oscillations of 1–10 mV amplitude persisted in the presence of 1 μM-TTX; these spontaneous depolarizations were abolished by 2 mM-cobalt ($n = 7$; Fig. 1) and by 0·5 and 1 mM-nickel ($n = 3$) but not by 0·1 mM-nickel ($n = 2$) nor ω-conotoxin (0·5 μM, $n = 2$).

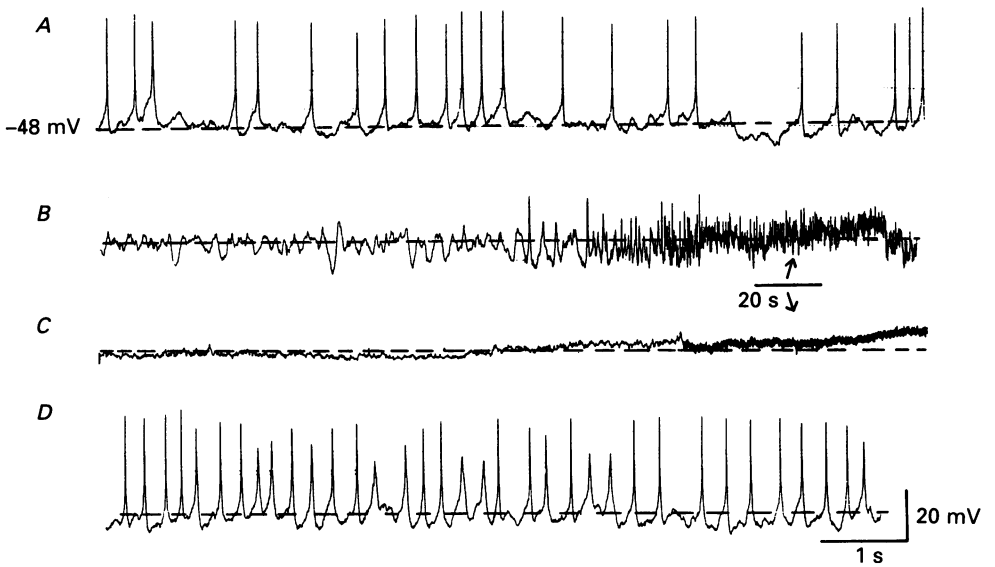

Fig. 1. Intracellular recording made from cultured melanotroph. Spontaneous action potential activity was abolished by application of 1 μM-TTX but small-amplitude membrane potential oscillations persisted. The addition of 2 mM-cobalt to the TTX-containing solution abolished these oscillations. Sections of the recording are shown in control (*A*), 3 min after the addition of TTX (1 μM) (*B*), 4 min after the addition of cobalt (2 mM) (*C*) and about 10 min after returning to normal solution (*D*).

Cadmium (30 and 100 μM) also abolished the TTX-insensitive spontaneous membrane potential oscillations ($n = 3$). Nifedipine (1 or 10 μM) irreversibly blocked TTX-insensitive spontaneous activity in four of seven cells but had no obvious action in the remainder. The average resting potential of spontaneously active cells was −43 ± 1·7 mV.

Initial resting potentials ranged from −35 to −50 mV when the whole cell configuration was used, and patch pipettes contained potassium salts (see Methods), but in the absence of any applied holding current, rapid and maintained depolarization occurred. This phenomenon has not been studied further; however, it was observed only when recordings were made at 34–37 °C. Resting potentials recorded at room temperature were stable for 30–60 min and averaged −54 ± 2·5 mV ($n = 13$; see also Taraskevich & Douglas, 1989). When the membrane was held at about −45 mV by applying 0·1–0·5 pA current through the patch pipette, repetitive action potentials occurred in 64% (sixteen of twenty-five) of cells. Small (1–8 mV) depolarizations were observed in the presence of TTX in these cells; they were abolished by 0·5 mM-cobalt ($n = 6$), 100 μM-cadmium ($n = 2$) and 500 μM nickel ($n = 3$). Input resistance (R_{in}) was measured in the presence of TTX and cobalt or

cadmium at a holding potential of −50 mV by applying 1–4 s duration hyperpolarizing current pulses of 1 pA amplitude; R_{in} ranged from 5 to 22 GΩ (12±1·4 GΩ, $n = 15$).

Effects of dopamine receptor agonists on membrane potential and current

Intracellular recordings

Superfusion with dopamine and dopamine agonists hyperpolarized thirty-two of thirty-five cells (90%) examined; dopamine (0·3 μM, $n = 6$), bromocryptine (0·1 μM,

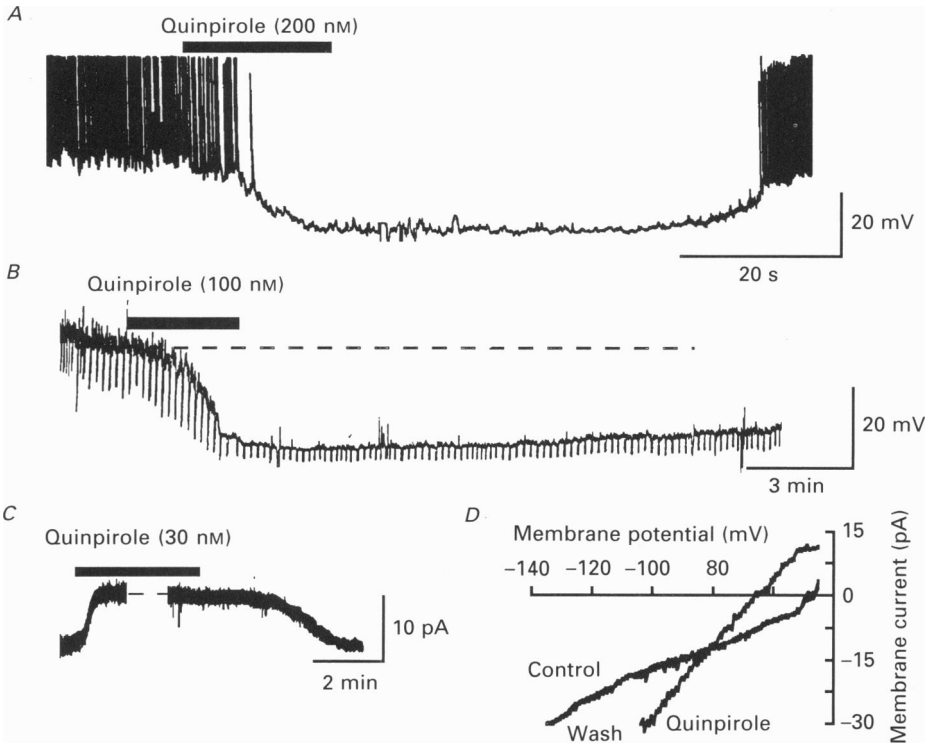

Fig. 2. Some properties of the potassium conductance increase evoked by quinpirole. *A*, superfusion with 200 nM-quinpirole (for duration indicated by filled bar) reversibly hyperpolarized the membrane by 26 mV; holding potential was −45 mV. *B*, hyperpolarization produced by quinpirole when the patch pipette contained 100 μM-GTP-γ-S; the effects of quinpirole did not reverse within 20 min from wash-out. Note difference in time scales between *A* and *B*. *C*, outward current evoked by quinpirole; holding potential, −45 mV. Dashed line indicates period of about 90 s during which current–voltage curve was obtained. *D*, current–voltage relations obtained from recording of *B* before, during and after superfusion with quinpirole; reversal potential was −84 mV with external potassium concentration of 6 mM.

$n = 12$) and quinpirole (0·1 μM, $n = 4$) produced membrane hyperpolarizations of 5–28 mV. The reversal potential for the dopamine-induced hyperpolarization was −104±2 mV ($n = 2$) and −87±4 mV ($n = 5$) when external potassium concentration was 2·5 and 5 mM respectively; these reversal potentials are consistent with a potassium-selective ionic conductance change. Similar results have been reported by Williams *et al.* (1989) for intact rat pituitary melanotrophs.

Whole-cell recordings

Figure 2 illustrates the actions of quinpirole on membrane potential and membrane current recorded with whole-cell patch electrodes. From a membrane potential of −45 mV, quinpirole hyperpolarized twenty-two of forty (55%) cells with threshold concentration and EC_{50} (half-maximal effective concentration) being 0·3 and 3 nM respectively (Figs 2*A* and 11*B*). The D_2 receptor antagonist, sulpiride (100 nM), reversibly abolished the hyperpolarization produced by supramaximal concentrations of quinpirole (0·3 and 1 μM; $n = 4$). The hyperpolarization was associated with a large decrease in the input resistance (Fig. 2*B*); in five cells control R_{in} was 10·1 ± 1 GΩ and in 100 nM-quinpirole R_{in} was 1·9 ± 0·5 GΩ.

Dopamine-mediated hyperpolarizations in melanotrophs have been shown previously to be abolished by pre-treatment with pertussis toxin (Williams *et al.* 1989), indicating the involvement of a pertussis-sensitive G-protein. Further support for G-protein coupling to dopamine hyperpolarization was obtained in the present study from experiments in which the non-hydrolysable GTP analogue, GTP-γ-S (100 μM), was included in the patch pipette. Hyperpolarizations in response to quinpirole (100 nM) were partially or completely irreversible ($n = 7$, Fig. 2*B*).

When membrane current was recorded under voltage-clamp conditions at −45 or −50 mV, quinpirole produced outward currents of 2–12 pA ($n = 19$, Fig. 2*C*). Such small currents would be expected in view of the very high input resistance of these cells; they also prevented construction of concentration–response curves to dopamine agonists or any quantitative analysis of the currents. Nevertheless, current–voltage curves obtained by applying ramp depolarizations from −120 to −45 mV showed a clear increase in slope conductance in the presence of quinpirole and a reversal from outward to inward current at approximately the potassium equilibrium potential (Fig. 2*D*).

Calcium currents

General properties

Figure 3*A* shows a series of current responses recorded from one cell in response to sequential 10 mV step depolarizations from the holding potential of −70 to 40 mV. Two components of this current were measured in this study, the initial peak, or transient current and the sustained current measured 10 ms prior to termination of the 150 ms duration voltage pulse. Both peak and sustained components activated at −40 mV and reached maximum amplitudes between 0 and 10 mV (Fig. 3*B*); maximum amplitudes of peak and sustained components were 370 ± 30 pA and 195 ± 20 pA with the ratio of peak to sustained components being 1·92 ± 0·1 ($n = 16$). Similar current–voltage relations were observed when the membrane potential was held at −30 mV or at −100 mV. No inward current was recorded when the membrane was held at −100 mV and stepped to −60 or −50 mV ($n = 5$).

Effects of calcium channel antagonists and agonists on calcium currents

Cadmium (1–500 μM) inhibited both components of the inward current to a similar degree at all voltages between −40 and 40 mV (Fig. 3*C* and *D*); 100 μM-cadmium completely and reversibly blocked all inward currents in all cells ($n > 40$; Fig. 3*C*). Nickel, at 100 μM, reduced the sustained component to a greater extent than the peak component (20 ± 5% inhibition of sustained *vs.* 8 ± 3% inhibition of peak,

$n = 5$) while a higher concentration (500 μM) inhibited peak current more than sustained current (68 ± 4 *vs.* 48 ± 5% inhibition respectively, $n = 5$; Fig. 4*A*). ω-Conotoxin (10–100 nM) did not alter inward currents during applications of up to 6 min ($n = 5$); a higher concentration (1 μM) reversibly inhibited the peak current by 25 ± 6% ($n = 7$) without changing the sustained component.

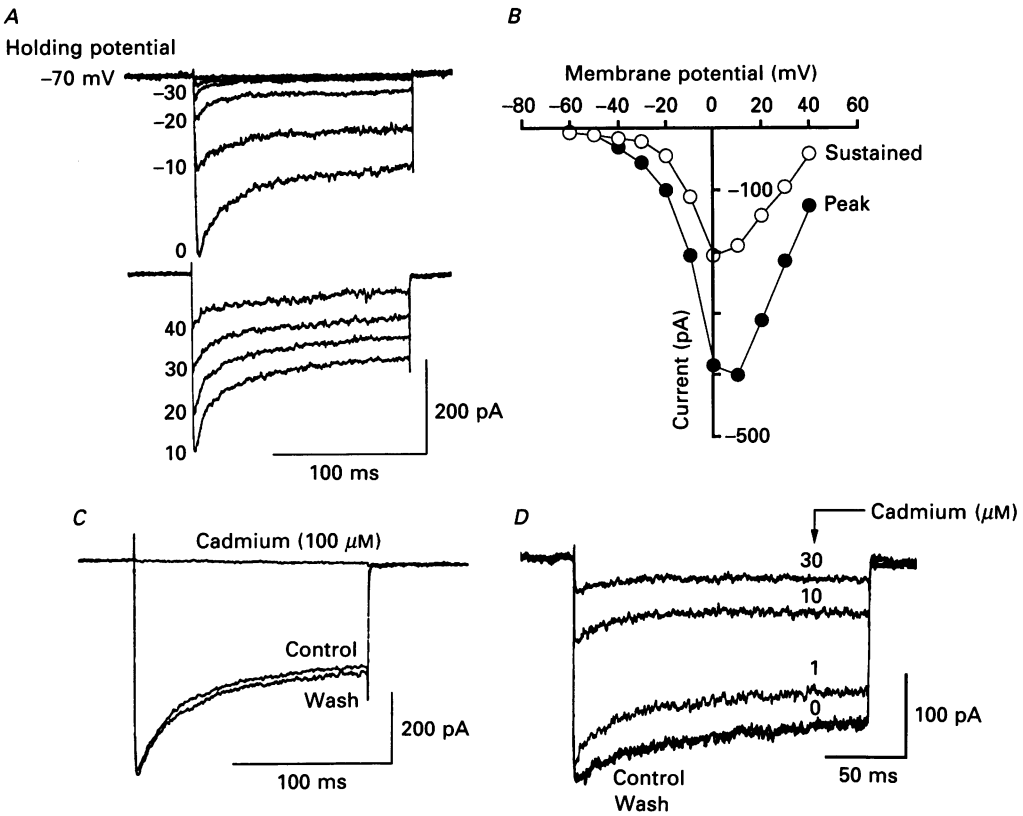

Fig. 3. Properties of calcium currents recorded from cultured melanotrophs. *A*, superimposed currents evoked by depolarizations from −70 mV to potentials indicated to the left of each trace. *B*, current-voltage relation obtained from records shown in *A*; peak and sustained components both activated at −40 mV. *C*, currents obtained by subtracting response in 500 μM-cadmium from that in other solutions; 100 μM-cadmium completely blocked the calcium current. *D*, inhibition of calcium current by 1, 10 and 30 μM-cadmium. Currents in *C* and *D* were evoked by depolarization from −70 to 0 mV. All recordings in this, and subsequent figures, were obtained at a temperature of 34–37 °C.

The actions of nifedipine on the calcium current evoked by stepping from −70 to 0 mV were examined in fourteen cells. Nifedipine partially reduced both components (Fig. 4*B*) with maximum inhibition occurring at a concentration of 10 μM (cf. Fig. 10*C*); this concentration reduced peak and sustained components by 31 ± 4 and 24 ± 8% respectively. Inhibition of peak current was not significantly different from that of sustained current at any concentration (0·1–10 μM).

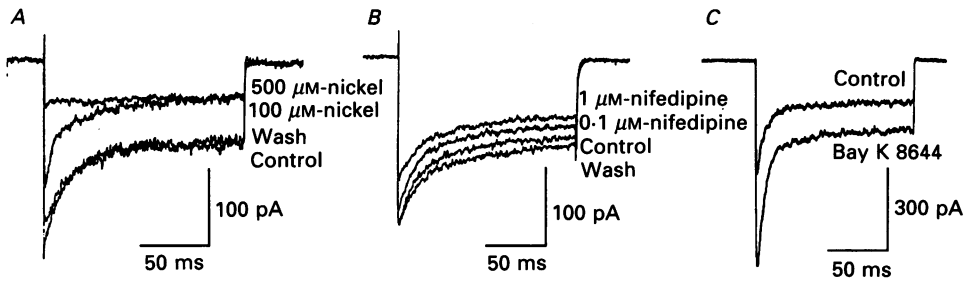

Fig. 4. Inhibition of calcium current by nickel (*A*) and nifedipine (*B*) and enhancement by Bay K 8644 (*C*). All traces are currents evoked by depolarizations from −70 to −10 mV; recordings in *A*–*C* are from three separate cells.

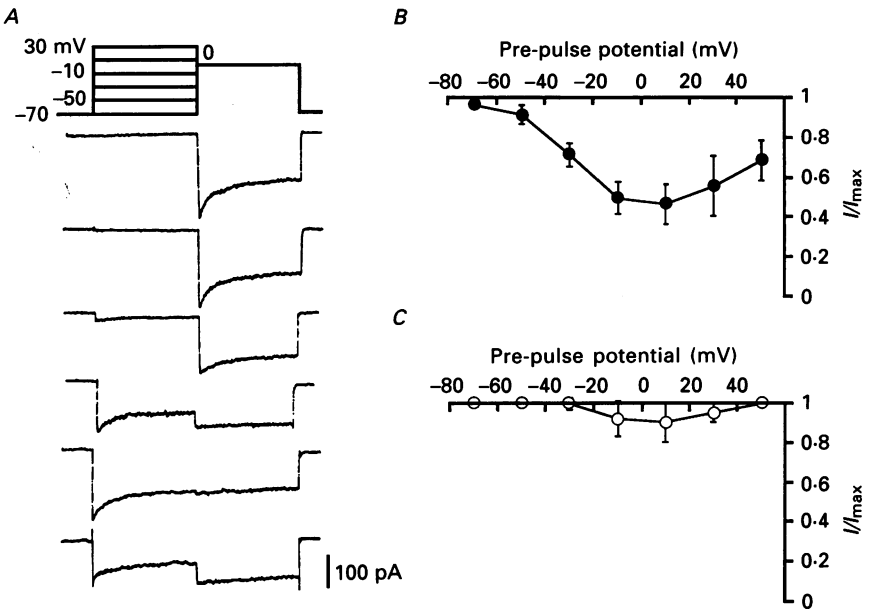

Fig. 5. Steady-state inactivation of calcium current. *A*, currents evoked in response to voltage protocol indicated in top panel; total duration of each trace is 350 ms. Average peak (*B*) and sustained (*C*) currents, as I/I_{max}, as a function of pre-potential; data were obtained from five separate experiments, three using voltage protocol shown in *A* and two in which an intervening 15 ms step back to −70 mV was applied prior to the test pulse.

Bay K 8644 (1 μM) increased both components of the calcium current (Fig. 4*C*). Average maximum increases in peak and sustained components were 139±1 and 126±9% ($n = 3$); these values were not significantly different ($P > 0{\cdot}07$).

Inactivation of calcium current

Inactivation of the calcium current was calculated from results obtained with 'double-pulse' voltage protocols of the type illustrated in Fig. 5. Two consecutive 150 ms duration voltage steps from a holding potential of −70 mV were applied with or without (Fig. 5*A*) an intervening 15 ms duration step back to −70 mV; the amplitude of the first pulse was incremented in 20 mV steps while the second pulse

was fixed at 0 mV. Similar results were obtained with either protocol and are plotted in Fig. 5*B* and *C*. The amplitude of the peak component evoked when the potential was stepped to 0 mV was more sensitive to the holding potential than was the sustained component. Depolarizing pre-pulses reduced the peak component by a

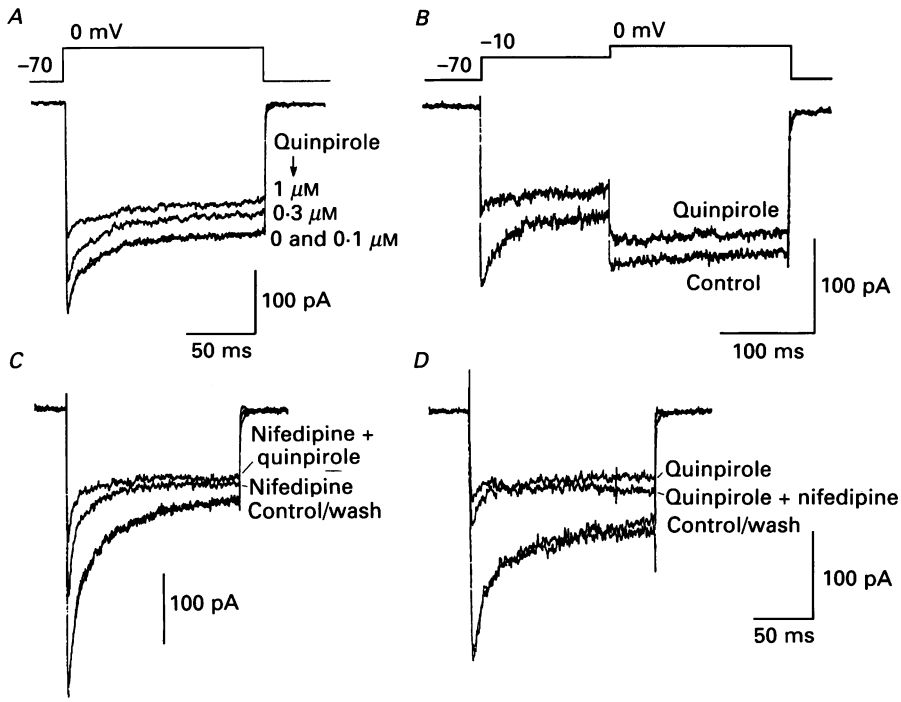

Fig. 6. Inhibition of calcium current by quinpirole. *A*, quinpirole (0·3 and 1 but not 0·1 μM) decreased current amplitude; higher concentrations did not produce further inhibition. *B*, quinpirole (1 μM) inhibited peak current to a greater extent than sustained current when the membrane was depolarized from −70 to −10 mV; when the membrane was further stepped to 0 mV the inhibition of peak and sustained currents was the same. *C*, nifedipine inhibited peak and sustained current by 36 and 20% respectively; quinpirole then inhibited this current by 27 and 8%. *D*, quinpirole inhibited peak and sustained currents by 53 and 33%; further addition of nifedipine had no appreciable effect. Recordings made in separate experiments; concentrations of nifedipine and quinpirole were 10 and 1 μM respectively.

maximum of 50% but reduced the sustained component by less than 10% (Fig. 5*B* and *C*).

Effects of quinpirole on calcium currents

Quinpirole inhibited calcium currents in all cells ($n = 49$) with maximum inhibition not exceeding 50% (Fig. 6*A*). Quinpirole reduced the peak component significantly more than the sustained current at all concentrations tested ($P < 0{\cdot}01$) with 3 μM-quinpirole producing maximum inhibition of peak and sustained current of $40 \pm 2{\cdot}5$% (cf. Fig. 10*A*) and 24 ± 3% respectively. Inhibition of the current was observed at potentials between −40 and 40 mV.

When the peak component was maximally inactivated by applying depolarizing pre-pulses, quinpirole still reduced the sustained component (Fig. 6*B*). Inhibition of the sustained component by quinpirole (1 μM) was not significantly reduced by depolarizing pre-pulses ($n = 3$). Experiments were also carried out in which

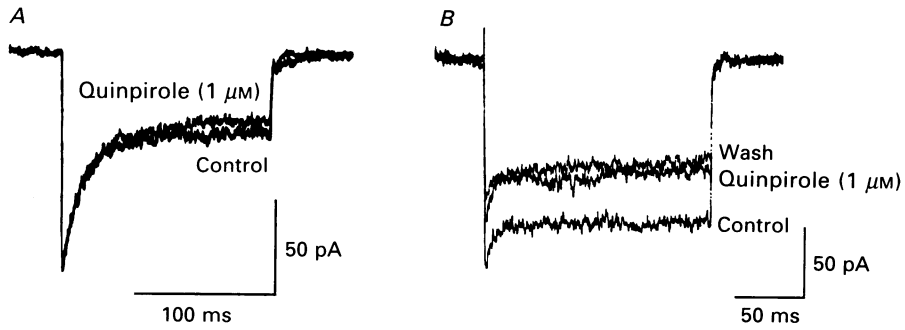

Fig. 7. Involvement of G-protein in calcium current inhibition by quinpirole. *A*, recording obtained in a preparation which had been incubated in pertussis toxin; quinpirole failed to inhibit the calcium current but always decreased the calcium current in untreated cells. *B*, recording obtained from one cell in which GTP-γ-S (100 μM) was added to the internal solution; the inhibition by quinpirole was irreversible. Recordings in *A* and *B* from separate experiments.

quinpirole and nifedipine were applied separately and then simultaneously in order to determine whether the inhibition was additive. When nifedipine (10 μM) was applied first (Fig. 6*C*), peak and sustained currents were reduced by 30 ± 3 and 24 ± 3% ($n = 3$). Addition of quinpirole at this time caused a significant ($P < 0{\cdot}01$) further reduction of these components (to 52 ± 8 and 33 ± 2% of control, Fig. 6*C*). However, when quinpirole (1 μM) was applied first, addition of nifedipine caused no further significant change in the calcium current ($n = 3$, Fig. 6*D*).

Involvement of G-proteins

GTP-γ-S (100 μM) was included in the recording pipette and calcium currents were recorded in the absence and presence of quinpirole (1 μM). In the majority of experiments (ten out of fifteen cells), quinpirole irreversibly reduced the current (Fig. 7*B*); in the remaining cells quinpirole continued to reversibly inhibit the current for the duration of the recording. Pre-treatment of cells with pertussis toxin (see Methods) abolished the action of quinpirole to reduce calcium currents ($n = 6$, Fig. 7*A*). These results are in accord with those obtained in anterior pituitary cells in which D_2 receptor-mediated inhibition of calcium currents has been shown to involve a pertussis-sensitive G-protein (Lledo, Legendre, Zhang, Israel & Vincent, 1990*a*).

β-Endorphin secretion

Basal hormone release

Basal levels of β-endorphin secretion from melanotrophs maintained in culture for 7–12 days were approximately 1% of cellular content per hour (Thomas *et al.* 1990); these rates of secretion (0·1–2% cellular hormone/h) are similar to those measured

in previous studies on both cultured and intact, denervated intermediate pituitary cells (Mains & Eipper, 1979; Eipper & Mains, 1980). The actions of TTX, calcium channel antagonists and quinpirole on basal hormone release were examined.

TTX (1 μM) did not alter basal hormone release (Fig. 8*A*, $n = 6$); similar results have been reported previously (Tomiko, Taraskevich & Douglas, 1984). Neither

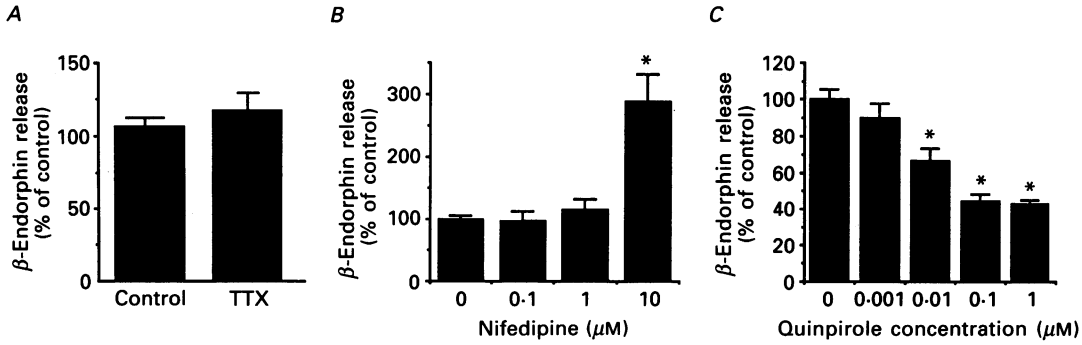

Fig. 8. Effects of TTX (*A*), nifedipine (*B*) and quinpirole (*C*) on basal rates of β-endorphin release. * = significantly different from control; $n = 6$–12 for each concentration.

cadmium (100 μM, $n = 12$) nor nickel (500 μM, $n = 6$) significantly altered basal rates of β-endorphin release ($P > 0{\cdot}2$) although a higher concentration of cadmium (1 mM) reduced basal secretion by $22 \pm 4\%$ ($n = 6$). Nifedipine (0·1 and 1 μM) also did not alter basal hormone release ($P > 0{\cdot}1$); however, 10 μM-nifedipine greatly enhanced hormone release by $265 \pm 36\%$ ($n = 8$, Fig. 8*B*).

Quinpirole reduced basal secretion in a concentration-dependent fashion (Fig. 8*C*); 100 nM-quinpirole produced maximum inhibition, reducing basal secretion to $44 \pm 4\%$ of control levels ($n = 6$). These results are similar to those previously reported for cultured neurointermediate lobes that were chronically treated with quinpirole (Gehlert, Bishop, Schafer & Chronwall, 1987).

Stimulated β-endorphin secretion

Two secretagogues were used in this study, the β-adrenergic receptor agonist, isoprenaline, and the calcium channel agonist, Bay K 8644 (see Cote, Munemura, Eskay & Kebabian, 1980; Taraskevich & Douglas, 1986). Isoprenaline (1 μM) and Bay K 8644 (1 μM) significantly increased hormone secretion to $165 \pm 7\%$ ($n = 39$) and $147 \pm 9\%$ ($n = 34$) of basal rates respectively (Fig. 9*A*). TTX (1 μM) did not alter secretion ($P > 0{\cdot}1$) evoked by isoprenaline ($n = 6$) or Bay K 8644 ($n = 5$).

Cadmium (10–100 μM) did not alter isoprenaline-stimulated secretion ($n = 18$) or Bay K 8644-stimulated secretion ($n = 18$, $P > 0{\cdot}1$; Fig. 10*A*). However, both 500 μM- and 1 mM-cadmium abolished stimulatory effects of Bay K 8644 ($n = 12$, Fig. 10*A*). Nickel (100 and 500 μM, $n = 6$ at each concentration) produced no significant changes in isoprenaline-stimulated secretion ($P > 0{\cdot}1$; Fig. 10*B*). Low concentrations of nifedipine (0·01, 0·1 μM) did not alter isoprenaline-induced stimulation of hormone release ($P > 0{\cdot}1$; $n = 12$) while higher concentrations (0·3 and 1 μM, $n = 12$) produced a small but insignificant ($P = 0{\cdot}03$) inhibition of stimulated release (Fig. 10*C*). β-

Endorphin secretion in the presence of 10 μM-nifedipine and 1 μM-isoprenaline was $434 \pm 71\%$ ($n = 6$) of control levels; this stimulation was significantly greater than stimulation by either isoprenaline (202%) or 10 μM-nifedipine (288%) alone.

Quinpirole reduced secretion evoked by either isoprenaline or Bay K 8644. The effect was concentration dependent with maximum inhibition of stimulated secretion

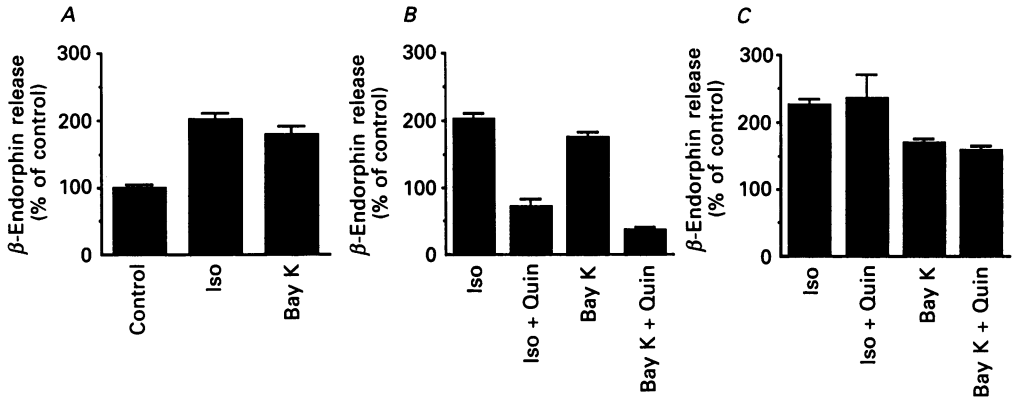

Fig. 9. Stimulation of β-endorphin release by isoprenaline (Iso) and Bay K 8644 (Bay K) (*A*) and its inhibition by quinpirole (Quin) (*B*, *C*). *A*, results from all experiments in which these agonists were used, $n = 48$. Both agonists consistently doubled secretion rates. *B*, quinpirole inhibited hormone release stimulated by both isoprenaline and Bay K 8644 ($n = 12$). *C*, results from identical experiments as in *B* but in preparations which had been treated with pertussis toxin. The agonist-induced stimulation was not altered but the inhibition by quinpirole was abolished. Concentrations of all substances were 1 μM throughout.

being observed at a concentration of 100 nM and EC_{50} concentration of 5–10 nM (Fig. 11*A*).

Stimulation produced by either isoprenaline or Bay K 8644 was not significantly altered by pertussis toxin pre-treatment ($n = 12$, Fig. 9*C*). In contrast, quinpirole-mediated inhibition of hormone release stimulated by either of these secretagogues was prevented by incubation of the cells with pertussis toxin ($n = 12$, Fig. 9*C*).

Correlations between secretion and ionic mechanisms

Figure 10 shows inhibition of peak calcium current and inhibition of stimulated β-endorphin secretion by cadmium, nickel and nifedipine. It is clear that concentrations of cadmium which abolished calcium currents did not alter isoprenaline or Bay K 8644-induced stimulation of hormone release (Fig. 10*A*). It can also be seen that, although cadmium produced a concentration-dependent inhibition of the calcium current, the inhibition of stimulated hormone release by high concentrations of cadmium was not concentration dependent. Similarly, concentrations of nickel which inhibited peak calcium current by about 65% (i.e. maximum effect of nickel) did not alter stimulated hormone release (Fig. 10*B*). Nifedipine, which inhibited calcium currents at all concentrations tested, had no effect on hormone release at concentrations of 1 μM and below but greatly stimulated secretion at 10 μM (Figs 8*B* and 10*C*).

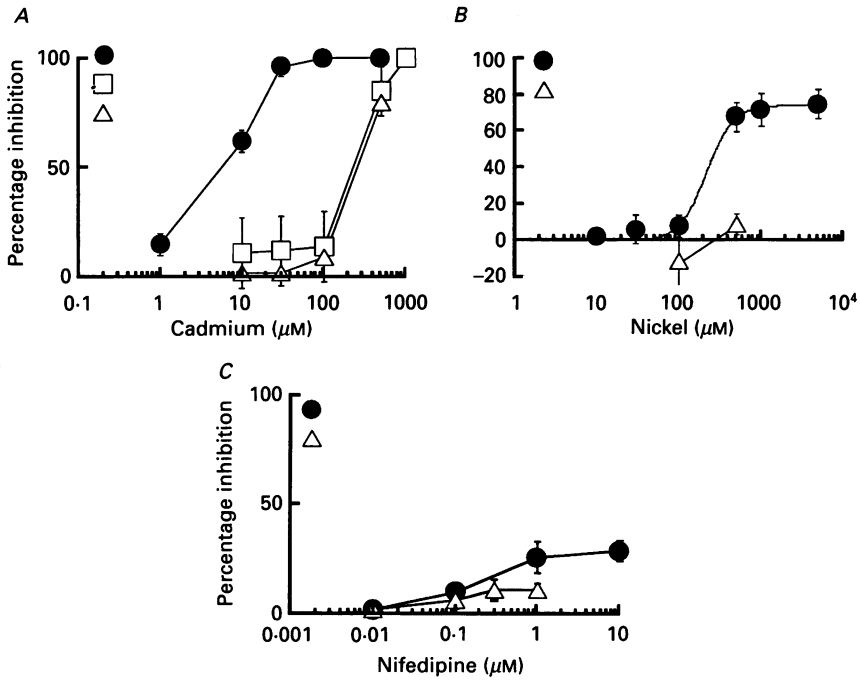

Fig. 10. Summary of effects of cadmium (*A*), nickel (*B*), and nifedipine (*C*) on peak calcium currents (●), and hormone release stimulated by Bay K 8644 (□) or isoprenaline (△). $n = 4$–10 for calcium currents, $n = 12$–24 for hormone release.

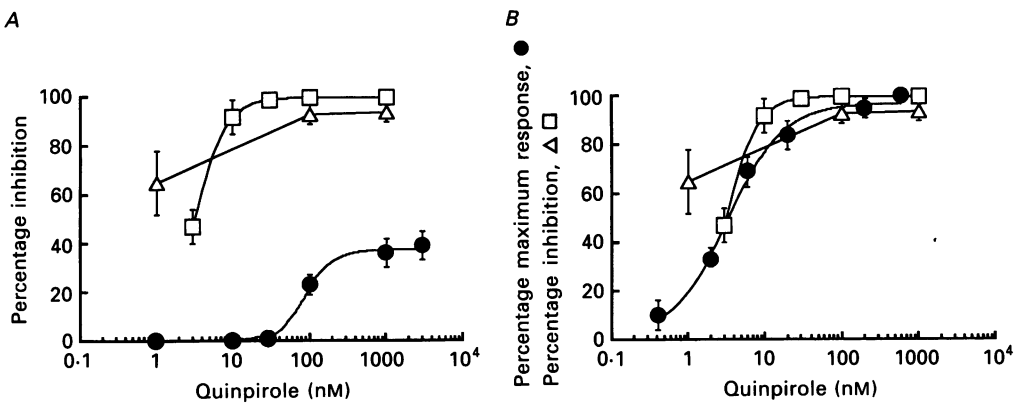

Fig. 11. Summary of actions of quinpirole to reduce calcium currents (*A*), to activate a potassium conductance (*B*) and to inhibit β-endorphin secretion (*A*, *B*). *A*, β-endorphin release stimulated by Bay K 8644 (□), or isoprenaline (△) and amplitude of peak calcium current (●) are plotted as a function of quinpirole concentration; each point for secretion is mean ± S.E.M. of twelve to fourteen experiments, each point for calcium current is from three to nine experiments. *B*, membrane hyperpolarization, as percentage of maximum hyperpolarization evoked in each cell (●), and hormone secretion (□, △) as in *A*. Each point for hyperpolarization is from six to fourteen experiments.

The actions of quinpirole on stimulated β-endorphin release, on calcium currents and on membrane potential are summarized in Fig. 11. Quinpirole abolished stimulated secretion at concentrations that were ineffective on peak (or sustained) calcium current (Fig. 11 *A*). In contrast, the concentration–hyperpolarization curve for the actions of quinpirole superimposed that for inhibition of stimulated hormone release (Fig. 11*B*).

DISCUSSION

Results from this study provide evidence *for* a relationship between activation of a potassium conductance by dopamine receptor activation and inhibition of hormone release; the most significant result in support of this conclusion was the superimposition of concentration–response curves for inhibition of β-endorphin secretion and membrane hyperpolarization by quinpirole. This study also provides evidence *against* a causal role for inhibition of hormone release resulting directly from a decrease in calcium current; the most convincing result in support of this conclusion was the finding that quinpirole produced maximum inhibition of hormone release at concentrations which did not alter the calcium current. Entirely unexpected was a converse finding that concentrations of calcium channel blockers which abolished calcium currents did not inhibit isoprenaline or Bay K 8644 stimulation of hormone release. How do these results relate to the stimulus–secretion coupling hypothesis of hormone release (Douglas, 1968; Douglas & Taraskevich, 1985)?

The calcium current

We could distinguish two overlapping components to the calcium current based on differences in pharmacology and differences in the voltage dependence of inactivation (but not activation) of the current. That is, the inward current evoked during a 150 ms depolarization showed both transient and sustained components. The transient, but not the sustained, component was readily inactivated by prior depolarization over the voltage range in which calcium entry occurs. We have made no attempt to examine mechanisms of inactivation of the calcium current in any detail; however, inactivation curves such as those obtained in the present study are usually characteristic of current-dependent, rather than voltage-dependent, inactivation (e.g. Eckert & Chad, 1984; but see also Jones & Marks, 1989). This transient component was more sensitive to inhibition by high concentrations of nickel (500 μM) and ω-conotoxin (1 μM) than was the sustained component. In some excitable cells, 10- to 100-fold lower concentrations of nickel selectively block a fast-inactivating low-threshold (LVA/T) calcium current (Carbone, Morad & Lux, 1987; Fox, Nowycky & Tsien, 1987), while in other tissues this current is not selectively altered (Carbone, Sher & Clementi, 1990). Similar results pertain to the selectivity of ω-conotoxin: nanomolar concentrations of this toxin irreversibly block an HVA/N (high-threshold, inactivating)-like current in some tissues but not in others (Aosaki & Kasai, 1989; Yoshikami, Bagabaldo & Olivera, 1989). The presence of an LVA/T current has been suggested in previous studies in cultured rat melanotrophs in which tail currents carried by barium ions were measured and in intact intermediate

pituitary cells in which intracellular electrodes were used to record calcium currents, but these studies did not examine effects of potentially selective blockers of the LVA/T current (Cota & Hiriart, 1989; Williams *et al.* 1990). Although the pharmacology and threshold for activation of the calcium current (about −40 mV for both peak and sustained components) observed in our experiments is more compatible with high-threshold, inactivating calcium currents (HVA/N currents, see Fox *et al.* 1987; Carbone *et al.* 1990), there is no reason to believe that the currents we recorded differ significantly from those described by Cota & Hiriart (1989) or Williams *et al.* (1990).

A dihydropyridine-sensitive, high voltage-activated (HVA/L) component was also present in all cells; based on inhibition by nifedipine this component comprised about 30% of total calcium current in melanotrophs. This finding is in agreement with electrophysiological studies carried out on all types of pituitary cells in which prominent HVA/L currents have been shown to exist (Hagiwara & Ohmori, 1982; Armstrong & Matteson, 1985; Mason & Sikdar, 1989; Lledo *et al.* 1990*a*; see McCleskey, Fox, Feldman & Tsien, 1986). It is interesting in this regard that calcium currents recorded from melanotrophs and other pituitary cells are very similar to calcium currents recorded from embryonic and neonatal neurones in possessing both dihydropyridine-sensitive and insensitive components (McCleskey *et al.* 1986; Carbone *et al.* 1987; Fox *et al.* 1987); adult mammalian neurones generally show minimal HVA/L currents (Schofield & Ikeda, 1988; Jones & Marks, 1989; Plummer, Logothetis & Hess, 1989; Surprenant, Shen, North & Tatsumi, 1990).

Do either of these calcium currents play a role in the TTX-insensitive spontaneous depolarizations that we and others (Tomiko *et al.* 1984) observed when membrane potential was recorded with intracellular microelectrodes? It was not possible to determine quantitative changes in these membrane oscillations because of their small amplitude and irregular frequency (e.g. Fig. 1); nevertheless, cadmium and nickel blocked the spontaneous membrane depolarizations at concentrations which maximally inhibited calcium currents and nifedipine blocked spontaneous oscillations in about half the cells over the concentration range which inhibited the HVA/L current. These results suggest that both components of the calcium current measured in our whole-cell recordings probably do underlie the calcium-dependent membrane potential oscillations. Such a conclusion has significant implications with regard to the role of these voltage-dependent calcium currents in hormone secretion.

Role of calcium currents in β-endorphin release

The calcium currents we recorded showed the same micromolar sensitivity to cadmium and nickel as do calcium currents present in embryonic and adult mammalian neurones (McCleskey *et al.* 1986; Jones & Marks, 1989; Surprenant *et al.* 1990), concentrations which produce parallel inhibition of neurotransmitter release in embryonic and adult neurones (Rane, Holz & Dunlap, 1987; Shen & Surprenant, 1990). Indeed, the tight correlation between concentration–response curves for inhibition of transmitter release and inhibition of calcium currents has provided evidence for the view that voltage-dependent calcium currents identified in neuronal cell bodies are identical to those responsible for transmitter release in nerve terminals (Rane *et al.* 1987; Lipscombe, Kongsamut & Tsien, 1989; Shen & Surprenant, 1990).

The stimulus–secretion coupling hypothesis of hormone release predicts similar results in pituitary cells.

There has long been incontrovertible evidence that depolarization-evoked (e.g. high potassium) hormone release from pituitary cells, in particular melanotrophs, requires an influx of extracellular calcium (for review see Douglas & Taraskevich, 1985). It is generally considered that the most likely entry-way is through voltage-dependent calcium channels; clearly, this study and many previous studies (e.g. Vale, Spiess, Rivier & Rivier, 1981; Taraskevich & Douglas, 1986; Mason & Sikdar, 1988; Lledo *et al.* 1990*a*), show that millimolar concentrations of cadmium and cobalt block stimulated hormone release in response to a number of secretagogues. Most studies of pituitary hormone release have not examined lower concentrations of calcium channel blockers; in our experiments, no inhibition of basal or stimulated hormone release occurred at concentrations of cadmium (100 μM) or nickel (500 μM) which maximally inhibited calcium currents at all membrane potentials. Similar discrepancies in concentrations of cadmium and nickel that blocked calcium currents (both LVA/T and HVA/L) and that inhibited prolactin release are apparent in a recent study in which the actions of dopamine in lactotrophs were examined (Lledo *et al.* 1990*a*). Nifedipine also failed to inhibit basal or stimulated hormone release in our experiments and in other studies dihydropyridine antagonists have produced inconsistent actions on pituitary hormone release (Taraskevich & Douglas, 1986). In a similar vein, several authors have puzzled over findings that TTX, which abolished the regularly occurring, high-amplitude spontaneous action potentials in melanotrophs and other pituitary cells and which must have simultaneously reduced depolarization-evoked calcium entry, did not alter basal or stimulated hormone release (Tomiko *et al.* 1984; Mason & Sikdar, 1988). It is difficult to reconcile such results with the view that these voltage-dependent calcium channels play the predominant role in stimulus–secretion coupling in pituitary cells.

On the other hand, Thomas *et al.* (1990) found a good correlation between depolarization-evoked calcium influx, apparently through voltage-dependent calcium channels, and exocytosis; in this study membrane capacitance was measured to monitor exocytosis from single melanotrophs. There is also one report in which adrenocorticotrophic hormone (ACTH) release from anterior pituitary corticotrophs was found to be inhibited by both TTX and micromolar concentrations of cadmium (Childs, Marchetti & Brown, 1987); in that study dihydropyridine antagonists blocked basal and stimulated ACTH release at 10–100-fold *lower* concentrations than those necessary to alter calcium currents. Further studies, in which the effects of excitatory and inhibitory secretagogues on calcium currents and exocytosis in single cells are examined, will be required in order to establish whether LVA and HVA calcium channels do provide the primary pathways for calcium influx in pituitary cells.

Actions of dopamine in melanotrophs

Quinpirole partially reduced both components of the calcium current; the maximum inhibition of the transient component was 40% while that of the sustained component was only 25% (e.g. Figs 5 and 10). The actions of quinpirole were abolished by pertussis toxin pre-treatment and were mimicked by intracellular

application of the nonhydrolysable GTP analogue, GTP-γ-S. Our results are essentially the same as those observed in a recent study in pituitary lactotrophs by Lledo *et al.* (1990*a*). Thus, it appears that activation of dopamine receptors on mammalian pituitary cells can lead to a direct, though partial, inhibition of both LVA/T and HVA/N/L currents through coupling to a pertussis-sensitive G-protein.

Whether or not voltage-dependent calcium currents are involved in stimulated hormone release (see above), it seems unlikely they are directly involved in inhibition of hormone release by dopamine. Concentrations of quinpirole (10–30 nM) that caused 90–100% inhibition of stimulated hormone release reduced calcium currents by less than 5%; at the EC_{50} concentration for inhibition of hormone release (5–10 nM) quinpirole did not alter the calcium current (Fig. 11). In neither our study nor that by Lledo *et al.* (1990*a*) did quinpirole or dopamine alter the time course of onset of the calcium current. In some neuronal cells, agonists which couple to inhibition of calcium currents through pertussis-sensitive G-proteins also alter the activation or inactivation kinetics and models in which small alterations in calcium current kinetics predict potentially large alterations in neurotransmitter release have been proposed in order to explain the agonist-induced abolition of transmitter release in the face of minimal inhibition in calcium current (Marchetti, Carbone & Lux, 1986; Bean, 1989; Elmslie, Zhou & Jones, 1990). But it is difficult to imagine how *no* observable change in the calcium current could be associated with up to 95% inhibition of hormone release.

Dopamine and quinpirole also produced membrane hyperpolarizations in melanotrophs; hyperpolarization was observed in most cells (90%) when intracellular microelectrodes were used but only in about half the cells in which whole-cell patch pipettes were used. This finding might suggest that an intracellular component necessary to couple the dopamine receptor to the potassium channel is readily lost during whole-cell dialysis. The actions of dopamine to hyperpolarize the membrane were similar to its actions at D_2 receptors in other mammalian pituitary cells (Israel, Kirk & Vincent, 1987; Lledo, Legendre, Zhang, Israel & Vincent, 1990*b*) and central neurones (Lacey, 1989; North, 1989) where the hyperpolarization results from activation of a potassium conductance, again involving a pertussis-sensitive G-protein. In contrast to the dissociation between inhibition of calcium current and inhibition of hormone release by quinpirole, membrane hyperpolarization occurred over the same concentration range which inhibited basal secretion and hormone release stimulated by both Bay K 8644 and isoprenaline; EC_{50} values for these actions were all about 5 nM (Fig. 11). We measured an average resting potential of -44 mV, approximately the threshold for activation of both transient and sustained components of the calcium current; at the quinpirole EC_{50} concentration the average membrane potential was -55 mV and only occasional TTX-sensitive and insensitive spontaneous depolarizations were observed. No spontaneous membrane oscillations were recorded at maximally effective concentrations of quinpirole (i.e. 100 nM) which hyperpolarized the membrane to about -60 mV. Such membrane potential effects would be expected to decrease or abolish calcium influx through voltage-dependent channels.

While dopamine D_2 receptors and other G-protein-coupled receptors have been shown in several studies to inhibit calcium currents and activate potassium

conductances by coupling to a pertussis-sensitive, cyclic AMP-independent pathway (Lacey, 1989; North, 1989), there is a third effector mechanism which may play a role in inhibition of hormone release from endocrine cells. Agonists such as somatostatin and noradrenaline can inhibit hormone secretion in permeabilized endocrine cells in a calcium-dependent, pertussis-sensitive, cyclic AMP-independent fashion, thus suggesting a site of action distal to both cyclic AMP second messenger systems and plasma membrane ion channels (Bittner, Holz & Neubig, 1986; Ullrich & Wollheim, 1988; Luini & DeMatteis, 1990). These types of studies have led to the hypothesis that receptor activation of a distinct G-protein, tightly associated with the exocytotic site, may directly alter vesicular fusion (Gomperts, 1986; Silinsky, 1986). The major difficulty in accepting this mechanism as playing a primary role in stimulus–secretion coupling is that levels of cytosolic calcium ($\geqslant 10\ \mu$M) required to stimulate secretion through this pathway (Ullrich & Wollheim, 1988; Luini & DeMatteis, 1990) are some 10-fold higher than those generally measured during stimulated secretion in intact cells (Guild, Itoh, Kebabian, Luini & Reisine, 1986; Thomas *et al.* 1990).

Concluding remarks

The primary aim of this study was to compare directly actions of dopamine D_2 receptor activation on membrane potential, ionic currents and hormone release in a single endocrine cell type under conditions as similar as possible. In summary, our results confirm previous findings that activation of the dopamine D_2 receptor both activates a potassium conductance and partially inhibits calcium currents in mammalian pituitary cells. Activation of the potassium conductance occurs over the same concentration range of quinpirole that inhibits basal and stimulated hormone release; inhibition of both LVA/T and HVA/LN calcium currents occurs only at quinpirole concentrations in excess of those that produce maximal inhibition of hormone release. We conclude that direct inhibition of voltage-dependent calcium currents by dopamine D_2 receptor activation is not the primary mechanism responsible for the inhibition of secretion in melanotrophs. Our data better support the conclusion that inhibition of calcium influx resulting from membrane hyperpolarization is the primary physiological mechanism underlying dopamine inhibition of hormone release. A less attractive but equally likely possibility is that the potassium conductance increase *per se* and ensuing hyperpolarization directly inhibits exocytotic hormone release.

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REFERENCES

ALLEN, R. G., HERBERT, E., HINMAN, M., SHIBUYA, H. & PERT, C. B. (1978). Coordinate control of corticotropin, β-lipotropin, and β-endorphin release in mouse pituitary cell cultures. *Proceedings of the National Academy of Sciences of the USA* **75**, 4972–4976.

AOSAKI, T. & KASAI, H. (1989). Characterization of two kinds of voltage-activated Ca-channel currents in chick sensory neurones. *Pflügers Archiv* **414**, 150–156.

ARMSTRONG, C. M. & MATTESON, D. R. (1985). Two distinct populations of calcium channels in a clonal line of pituitary cells. *Science* **227**, 65–68.

BEAN, B. P. (1989). Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature* **340**, 153–156.

BEAULIEU, M., FELDER, R. & KEBABIAN, J. W. (1986). D_2 dopaminergic agonists and adenosine 3′,5′-monophosphate directly regulate the synthesis of α-melanocyte-stimulating hormone-like peptides by cultured rat melanotrophs. *Endocrinology* **118**, 1032–1039.

BITTNER, M. A., HOLZ, R. W. & NEUBIG, R. R. (1986). Guanine nucleotide effects on catecholamine secretion from digitonin-permeabilized adrenal chromaffin cells. *Journal of Biological Chemistry* **261**, 10182–10188.

CARBONE, E., MORAD, M. & LUX, H. D. (1987). External Ni^{2+} selectively blocks the low threshold Ca^{2+} current of chick sensory neurons. *Pflügers Archiv* **408**, suppl. R60.

CARBONE, E., SHER, E. & CLEMENTI, F. (1990). Ca currents in human neuroblastoma IMR32 cells: kinetics, permeability and pharmacology. *Pflügers Archiv* **416**, 170–179.

CHILDS, G. V., MARCHETTI, C. & BROWN, A. M. (1987). Involvement of sodium channels and two types of calcium channels in the regulation of adrenocorticotropin release. *Endocrinology* **120**, 2059–2069.

COTA, G. & HIRIART, M. (1989). Hormonal and neurotransmitter regulation of Ca channel activity in cultured adenohypophyseal cells. In *Secretion and its Control*, ed. OXFORD, G. S. & ARMSTRONG, C. M., pp. 143–165. Rockefeller University Press, New York.

COTE, T., MUNEMURA, M., ESKAY, R. L. & KEBABIAN, J. W. (1980). Biochemical identification of the β-adrenoceptor and evidence for the involvement of an adenosine 3′,5′-monophosphate system in the β-adrenergic induced release of α-melanocyte-stimulating hormone in the intermediate lobe of the rat pituitary gland. *Endocrinology* **107**, 108–116.

DOUGLAS, W. W. (1968). Stimulus–secretion coupling: the concept and clues from chromaffin and other cells. *British Journal of Pharmacology* **34**, 451–474.

DOUGLAS, W. W. & TARASKEVICH, P. S. (1985). The electrophysiology of adenohypophyseal cells. In *The Electrophysiology of the Secretory Cell* ed. POISNER, A. M. & TRIFARO, J. M., pp. 63–92. Elsevier, Amsterdam.

ECKERT, R. & CHAD, J. E. (1984). Inactivation of Ca channels. *Progress in Biophysics and Molecular Biology* **44**, 215–267.

EIPPER, B. A. & MAINS, R. E. (1980). Structure and biosynthesis of proadreno-corticotropin and related peptides. *Endocrine Reviews* **1**, 1–26.

ELMSLIE, K. S., ZHOU, W. & JONES, S. W. (1990). LHRH and GTP-γ-S modify calcium current activation in bullfrog sympathetic neurons. *Neuron* **5**, 75–80.

FOX, A. P., NOWYCKY, M. C. & TSIEN, R. W. (1987). Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *Journal of Physiology* **394**, 149–172.

GEHLERT, D. R., BISHOP, J. F., SCHAFER, M. P. & CHRONWALL, B. M. (1987). Rat intermediate lobe in culture: dopaminergic regulation of POMC biosynthesis and cell proliferation. *Peptides* **8**, 1–8.

GOMPERTS, B. D. (1986). Calcium shares the limelight in stimulus–secretion coupling. *Trends in Biochemical Sciences* **11**, 290–292.

GUILD, S., ITOH, Y., KEBABIAN, J. W., LUINI, A. & REISINE, T. (1986). Forskolin enhances basal and potassium-evoked hormone release from normal and malignant pituitary cells: the role of calcium. *Endocrinology* **118**, 268–279.

HAGIWARA, S. & OHMORI, H. (1982). Studies of calcium channels in rat clonal pituitary cells with patch electrode voltage clamp. *Journal of Physiology* **331**, 231–252.

HATFIELD, J. M., ALLEN, R. G., STACK, J. & RONNEKLEIV, O. (1988). Post-translational processing of pro-opiomelanocortin (POMC)-derived peptides during fetal monkey pituitary development. II. β-lipotropin (β-LPH)-related peptides. *Developmental Biology* **126**, 164–172.

ISRAEL, J. M., KIRK, C. & VINCENT, J. D. (1987). Electrophysiological responses to dopamine of rat hypophyseal cells in lactotroph-enriched primary cultures. *Journal of Physiology* **390**, 1–22.

JOHNSON, J. W. & ASCHER, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* **325**, 529–531.

JONES, S. W. & MARKS, T. N. (1989). Calcium currents in bullfrog sympathetic neurons. II. Inactivation. *Journal of General Physiology* **94**, 169–182.

LACEY, M. G. (1989). Towards understanding dopamine. *Seminars in the Neurosciences* **1**, 137–144.

LIPSCOMBE, D., KONGSAMUT, S. & TSIEN, R. W. (1989). α-Adrenergic inhibition of sympathetic neurotransmitter release mediated by modulation of N-type calcium channel gating. *Nature* **340**, 639–642.

LLEDO, P., LEGENDRE, P., ZHANG, J., ISRAEL, J. & VINCENT, J. (1990*a*). Dopamine inhibits two characterized voltage-dependent calcium currents in identified rat lactotroph cells. *Endocrinology* **127**, 990–1001.

LLEDO, P., LEGENDRE, P., ZHANG, J., ISRAEL, J. & VINCENT, J. (1990*b*). Effects of dopamine on voltage-dependent potassium currents in identified rat lactotrophs. *Neuroendocrinology* (in the Press).

LUINI, A. & DEMATTEIS, M. A. (1990). Evidence that receptor-linked G protein inhibits exocytosis by a post-second messenger mechanism in ATt20 cells. *Journal of Neurochemistry* **54**, 30–38.

MCCLESKEY, E. W., FOX, A. P., FELDMAN, D. & TSIEN, R. W. (1986). Different types of calcium channels. *Journal of Experimental Biology* **124**, 177–190.

MAINS, R. E. & EIPPER, B. A. (1979). Synthesis and secretion of corticotropins, melanotropins, and endorphins by rat intermediate pituitary cells. *Journal of Biological Chemistry* **254**, 7885–7894.

MARCHETTI, C., CARBONE, E. & LUX, H. D. (1986). Effects of dopamine and noradrenaline on Ca channels of cultured sensory and sympathetic neurons of chick. *Pflügers Archiv* **406**, 104–111.

MASON, W. T. & SIKDAR, S. K. (1988). Characterization of voltage-gated sodium channels in ovine gonadotrophs: relationship to hormone secretion. *Journal of Physiology* **399**, 493–517.

MASON, W. T. & SIKDAR, S. K. (1989). Characteristics of voltage-gated Ca^{2+} currents in ovine gonadotrophs. *Journal of Physiology* **415**, 367–391.

MIYAZAKI, K., GOLDMAN, M. E. & KEBABIAN, J. W. (1984). Forskolin stimulates adenylate cyclase activity, adenosine 3′,5′-monophosphate production and peptide release from the intermediate lobe of the rat pituitary gland. *Endocrinology* **114**, 761–766.

MUNEMURA, M., ESKAY, R. L. & KEBABIAN, J. W. (1980). Release of α-melanocyte stimulating hormone from dispersed cells of the intermediate lobe of the rat pituitary gland: involvement of catecholamines and adenosine 3′,5′-monophosphate. *Endocrinology* **106**, 1795–1803.

NICOLL, R. A., MALENKA, R. C. & KAUER, J. A. (1990). Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiological Reviews* **70**, 513–566.

NORTH, R. A. (1989). Drug receptors and the inhibition of nerve cells. *British Journal of Pharmacology* **98**, 13–28.

PLUMMER, M. R., LOGOTHETIS, D. E. & HESS, P. (1989). Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. *Neuron* **2**, 1453–1463.

RANE, S. G., HOLZ, G. G. & DUNLAP, K. (1987). Dihydropyridine inhibition of neuronal calcium current and substance P release. *Pflügers Archiv* **409**, 361–366.

REES, L. H., COOK, D. M., KENDALL, J. W., ALLEN, C. F., KRAMER, R. M., RATCLIFFE, J. G. & KNIGHT, R. A. (1971). A radioimmunoassay for rat plasma ACTH. *Endocrinology* **89**, 254–261.

SCHOFIELD, G. G. & IKEDA, S. R. (1988). Sodium and calcium currents of acutely isolated adult rat superior cervical ganglion neurons. *Pflügers Archiv* **411**, 481–490.

SHEN, K.-Z. & SURPRENANT, A. (1990). Mechanisms underlying presynaptic inhibition through α_2-adrenoceptors in guinea-pig submucosal neurones. *Journal of Physiology* **431**, 609–628.

SILINSKY, E. M. (1986). Inhibition of transmitter release by adenosine: are Ca^{2+} currents depressed or are the intracellular effects of Ca^{2+} impaired? *Trends in Pharmacological Sciences* **7**, 180–185.

STACK, J. (1990). The regulation of voltage-dependent calcium currents and their role in modulation of stimulus–secretion coupling in pituitary. Ph.D. Thesis, Oregon Health Sciences University, OR, USA.

STACK, J., SURPRENANT, A. & ALLEN, R. G. (1987). Dopamine increases a K^+ conductance and inhibits spontaneous action potentials recorded from melanotrophs of the rat pituitary. *Society for Neurosciences Abstracts* **13**, 532.

STACK, J., ZLATNICK, M., ALLEN, R. G. & SURPRENANT, A. (1990). Dopamine D_2 receptor activation modulates secretion and calcium currents in a dose-dependent but non-correlative manner. *Abstracts of the 72nd Annual Meeting*, p. 262. The Endocrine Society, Bethesda, MD, USA.

SURPRENANT, A., SHEN, K.-Z., NORTH, R. A. & TATSUMI, H. (1990). Inhibition of calcium currents by noradrenaline, somatostatin and opioids in guinea-pig submucosal neurones. *Journal of Physiology* **431**, 585–608.

TARASKEVICH, P. S. & DOUGLAS, W. A. (1986). Effects of Bay K 8644 and other dihydropyridines on basal and potassium-evoked output of MSH from mouse melanotrophs *in vitro*. *Neuroendocrinology* **44**, 384–389.

TARASKEVICH, P. S. & DOUGLAS, W. W. (1989). Effects of Bay K 8644 on Ca-channel currents and electrical activity in mouse melanotrophs. *Brain Research* **491**, 102–108.

THOMAS, P., SURPRENANT, A. & ALMERS, A. (1990). Cytosolic Ca^{2+}, exocytosis and endocytosis in single melantrophs of the rat pituitary. *Neuron* (in the Press).

TOMIKO, S. A., TARASKEVICH, P. S. & DOUGLAS, W. W. (1984). Effects of veratridine, tetrodotoxin and other drugs that alter electrical behavior on secretion of melanocyte-stimulating hormone from melanotrophs of the pituitary pars intermedia. *Neuroscience* **12**, 1223–1228.

ULLRICH, A. & WOLLHEIM, C. B. (1988). GTP-dependent inhibition of insulin secretion by epinephrine in permeabilized RINm5F cells. *Journal of Biological Chemistry* **263**, 8615–8620.

VALE, W., SPIESS, J., RIVIER, C. & RIVIER, J. (1981). Characterization of a 41 residue ovine hypothalamic peptide that stimulates the secretion of corticotropin and β-endorphin. *Science* **213**, 1394.

WILLIAMS, P. J., MACVICAR, B. A. & PITTMAN, Q. J. (1989). A dopaminergic inhibitory postsynaptic potential mediated by an increased potassium conductance. *Neuroscience* **31**, 673–681.

WILLIAMS, P. J., MACVICAR, B. A. & PITTMAN, Q. J. (1990). Synaptic modulation by dopamine of calcium currents in rat pars intermedia. *Journal of Neuroscience* **10**, 757–763.

YOSHIKAMI, D., BAGABALDO, Z. & OLIVERA, B. M. (1989). The inhibitory effects of ω-conotoxins on Ca channels and synapses. *Annals of the New York Academy of Sciences* **560**, 230–248.