# MODULATION OF SECRETION BY DOPAMINE INVOLVES DECREASES IN CALCIUM AND NICOTINIC CURRENTS IN BOVINE CHROMAFFIN CELLS

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## SUMMARY

1. Catecholamine secretion from cultured bovine adrenal chromaffin cells was decreased in a dose-dependent manner by the $D_2$ dopamine agonists apomorphine and LY 17 1555.

2. $^{45}Ca^{2+}$ uptake was similarly inhibited and whole-cell $Ca^{2+}$ currents were reduced by apomorphine.

3. These inhibitory effects of $D_2$ agonists depended on the secretagogue used, being much more pronounced for nicotine-evoked responses compared to high $K^+$ stimulation, indicating another possible site of action of apomorphine up-stream of $Ca^{2+}$ entry.

4. Inhibition by apomorphine of nicotine-evoked responses could not be explained by competitive antagonism against nicotine or DMPP (1,1-dimethyl-4-phenyl-piperazinium iodide).

5. Apomorphine caused reductions of inward whole-cell nicotinic current evoked by ACh and nicotine.

6. Inhibition of nicotine-evoked secretion and $^{22}Na^+$ influx by apomorphine were not affected by tetrodotoxin, and voltage-dependent, whole-cell $Na^+$ currents were unaltered by apomorphine.

7. No evidence was obtained for increases in $K^+$ conductance by apomorphine.

8. Action potentials recorded in whole-cell current clamp were blocked by apomorphine when they were triggered by nicotinic depolarization but not when they were elicited by direct electrical stimulation.

9. Inclusion of GDP-$\beta$-S in the pipette internal solution did not affect apomorphine-dependent inhibition of nicotinic-evoked responses, while the decrease in whole-cell $Ca^{2+}$ current induced by apomorphine was completely inhibited in the presence of GDP-$\beta$-S.

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10. Increases in cyclic AMP caused by cholera toxin and forskolin did not change the apomorphine-dependent inhibitory effects on nicotine-evoked secretion, indicating that changes in cyclic AMP levels caused by dopamine receptor stimulation are probably not involved.

## INTRODUCTION

The adrenal medulla is embryologically derived from the neural crest and shares many properties in common with sympathetic neurones, including the ability to synthesize catecholamines from L-tyrosine. Dopamine is produced during this biosynthetic process, and while it has generally been considered that dopamine is used only for the synthesis of catecholamines, evidence obtained from a variety of peripheral nervous system tissues indicates that dopamine may have a functional role in the adrenal medulla (Bell, 1982; Goldberg & Kohli, 1983; Lackovic & Neff, 1983).

The presence of dopamine receptors in adrenomedullary chromaffin cells has been widely reported. Dopaminergic agonists were shown to inhibit secretion of adrenaline and noradrenaline from perfused adrenal glands (Artalejo, Garcia, Montiel & Sanchez-Garcia, 1985; Gonzalez, Artalejo, Montiel, Hervas & Garcia, 1986) and from chromaffin cells maintained in primary culture (Bigornia, Suozzo, Ryan, Napp & Schneider, 1988). The reversal of the inhibitory effects of dopamine by specific $D_2$ antagonists suggests that the effects are mediated by a $D_2$ receptor in the adrenal medulla and the presence of $D_2$ but not $D_1$ dopamine receptors was demonstrated in ligand binding studies on chromaffin cell membrane suspensions (Gonzales *et al.* 1986; Lyon, Titeler, Bigornia & Schneider, 1987; Quick, Bergeron, Mount & Philie, 1987). Thus, the presence and the role of $D_2$ receptors on adrenal medullary chromaffin cells seem to be well characterized, but the precise mechanisms underlying the inhibitory action of dopamine remain to be elucidated.

While earlier work demonstrated that dopamine inhibited nicotinic-evoked catecholamine release (Artalejo *et al.* 1985), more recent data suggest that dopamine decreases secretion from adrenal chromaffin cells by altering $Ca^{2+}$ channel conductance (Bigornia *et al.* 1988). To assess the possible role of voltage-gated $Ca^{2+}$ channels and of acetylcholine-activated channels in the dopamine-induced inhibition of catecholamine release, we have compared the effect of $D_2$ agonists on $Ca^{2+}$ and $Na^+$ uptake evoked by different secretagogues. Also, the effects of apomorphine on ionic currents through voltage-sensitive $Ca^{2+}$, $Na^+$ and $K^+$ channels and through the channels associated with nicotinic receptors were directly measured using whole-cell patch clamp techniques. The observations presented here indicate that voltage-dependent $Ca^{2+}$ channels are not solely responsible for dopamine-induced inhibition of catecholamine release since current through nicotinic receptor associated channels is also decreased.

## METHODS

### Primary culture of chromaffin cells

The procedure for isolating and culturing bovine chromaffin cells has been described previously (Bader, Trifaro, Langley, Thiersé & Aunis, 1986). Cells were grown on twenty-four multiple 16 mm well Costar plates (Cambridge, MA, USA) at a density of $2.5 \times 10^5$ cells/well for [³H]noradrenaline ([³H]NA) release experiments and of $5 \times 10^5$ cells/well for $^{45}Ca^{2+}$ and $^{22}Na^+$ uptake experiments. Cells

were maintained at 37 °C in a humidified atmosphere containing 5 % $CO_2$, 95 % air. All secretion and uptake experiments were carried out at 37 °C on cultures 2–7 days after plating.

*Catecholamine secretion*

Chromaffin cells in primary culture were pre-loaded with 125 nM [³H]NA for 60 min in a culture medium containing no amino acids. This was followed by four washes with Locke solution (which contained in mM: NaCl, 140; KCl, 4·7; $CaCl_2$, 2·5; $KH_2PO_4$, 1·2; $MgSO_4$, 1·2; EDTA, 0·01; glucose, 11; ascorbic acid, 0·56; HEPES, 15; pH 7·5). Washing intervals were set constant at 10 min. Chromaffin cells were then incubated for 10 min in Locke solution containing variable amounts of apomorphine or LY 17 1555, another specific $D_2$ agonist. Stimulation was induced by incubating cells in Locke solution containing either 20 µM-nicotine, 50 µM-veratridine or 59 mM-KCl (made by decreasing NaCl isosmotically). [³H]NA release was determined in the incubation media following stimulation by measuring the radioactivity present in the incubation media after centrifugation for 10 min at $12000 \times g$ ($X$) and in the cells after precipitation with 10 % TCA ($Y$). The quantity of catecholamine release is expressed as the percentage of the total radioactivity present in the cells prior to stimulation as determined by $[X/(X+Y)]100$. Net secretory values, obtained by subtraction of basal levels, were used in the calculation of the percentage inhibition produced by apomorphine.

*⁴⁵Ca²⁺ and ²²Na⁺ uptake measurements*

Measurements of $^{45}Ca^{2+}$ and $^{22}Na^+$ influxes were performed using previously described methods (Amy & Kirshner, 1982; Wada, Yashima, Izumi, Kobayashi & Yanagihara, 1984; Artalejo, Garcia & Aunis, 1987).

Briefly, for $^{45}Ca^{2+}$ uptake measurements, chromaffin cells first were washed several times with Locke solution to remove the culture medium, and then incubated for 10 min in Locke solution containing increasing concentrations of apomorphine. Cells were subsequently stimulated for 30 s with Locke solution containing either 20 µM-nicotine or 59 mM-K⁺ (NaCl replaced isosmotically by KCl) and $^{45}Ca^{2+}$ (35·12 mCi/mg) at a final concentration of 1 µCi/0·2 ml. After the 30 s stimulation, the medium was removed rapidly and cells were washed (six times at 10 s intervals) with Ca²⁺-free Locke solution with 3·7 mM-$MgSO_4$, 2 mM-EGTA and 2 mM-$LaCl_3$, in order to stop the uptake reaction and to eliminate aspecific binding of Ca²⁺ to the plasma membrane. Cells were then precipitated with 10 % TCA and the radioactivity remaining in the cells was estimated.

For $^{22}Na^+$ uptake experiments, chromaffin cells first were washed several times with Locke solution in the presence or absence of apomorphine. Cells were subsequently stimulated for 5 min with Locke solution containing either 20 µM-nicotine, 59 mM-K⁺, or 50 µM-veratridine and 1 µCi/0·2 ml $^{22}Na^+$ (32 mCi/mg). Incubation and stimulation were performed in Locke solution containing 50 mM-NaCl and 180 mM-sucrose, in order to reduce the ratio between radioactive and non-radioactive Na⁺ (Amy & Kirshner, 1982; Wada *et al.* 1984). During stimulation, ouabain (1 mM) was also included to block the activity of the Na⁺–K⁺-ATPase (Amy & Kirshner, 1982; Wada *et al.* 1984). Cells were then washed three times with calcium-free Locke solution, in order to stop the uptake reaction, and radioactivity was measured in the cells after precipitation with 10 % TCA using an LKB γ-ray counter. For the calculation of percentage inhibition by apomorphine, net influx values, obtained by subtraction of basal levels, were used.

*Electrophysiology*

Standard whole-cell patch clamp techniques were used (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The normal external bathing solution contained (in mM): NaCl, 135; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; glucose, 11; HEPES, 10; pH 7·3 with NaOH. Patch pipettes had resistances of 2–5 MΩ when filled. Pipettes were coated with beeswax and dipped in Sigmacote (Sigma) to minimize associated capacitive transients. Internal solutions for recording voltage-gated K⁺ and nicotinic agonist-activated currents, and action potentials under current clamp, contained (in mM): KCl, 135; $MgCl_2$, 2; $Na_2ATP$, 5; EGTA, 11/$CaCl_2$, 1 (pCa 8); HEPES, 20; pH 7·3 with KOH. Occasionally, the internal Ca²⁺ buffer was BAPTA, 5·5 mM/$CaCl_2$ 0·5 mM (pCa 8). For measurements of voltage-dependent Na⁺ and Ca²⁺ currents, KCl and KOH were replaced with CsCl and CsOH, respectively and 20 mM-TEA-Cl added in the internal solution, in order to block K⁺ currents. In some experiments, as indicated, 200 µM-GTP or GDP-β-S were included in the internal solutions. Tetrodotoxin (TTX; 10 µM) or 1 mM-$CdCl_2$, and 20 mM-TEA-Cl were added to the

external solution to block $Na^+$ or $Ca^{2+}$ and $K^+$ currents respectively, when appropriate. Drugs were made as concentrated solutions, aliquoted and stored at $-20$ °C. Dilutions were made daily, into normal external sodium solution. To minimize degradation, apomorphine solutions were protected from light, and apomorphine puffer pipettes were painted black.

For the dopaminergic effects on nicotinic current, either agonist alone (acetylcholine or nicotine), or agonist together with apomorphine, were locally microperfused from separate, pressurized puffer pipettes positioned with their tips close together ($< 20 \mu$m apart), both about 30 $\mu$m from the cell. Note that both pipettes contained the same agonist concentration. Puffer pressure and distance from the cell were adjusted so that maintained nicotinic currents were obtained. In order to minimize the implications of nicotinic receptor desensitization, a period of 3 min separated control applications of agonist from trials with apomorphine. With this interval, stable successive responses to agonist alone were routinely observed. After trials with apomorphine, recovery was assessed following wash-out for 3 min. For dopaminergic effects on voltage-activated currents, a single, apomorphine-containing puffer pipette was used, and apomorphine was applied usually for 1–2 min. Control applications of external solution only had no effect on either nicotinic or voltage-activated currents.

Series resistance and capacitance compensation were made using the inbuilt circuitry of the patch amplifier (List EPC/7; Darmstadt, FRG). A holding potential of $-90$ mV was used throughout. Step voltage commands (10 or 35 ms long) were given every 3 and 10 or 20 s, for $Na^+$ and $Ca^{2+}$ currents, respectively. Run-down of $Ca^{2+}$ current was thus minimized, being $\leqslant 5$ % over 20 min in the best cases. Agonist-mediated decreases in $Ca^{2+}$ and nicotinic currents were considered only if recovery to at least 90 % of control values (or clearly, to the initial run-down slope for $Ca^{2+}$ current) was observed upon wash-out. Voltage ramp commands were used to obtain quasi-steady-state $I$–$V$ curves. Apomorphine action on resting and action potentials was examined under whole-cell current clamp. Data were recorded on FM or videotape, analysed off-line with the aid of a digital oscilloscope after appropriate filtering and plotted using an $X$–$Y$ plotter. All the records shown are non-leak-subtracted. Experiments were made at room temperature (20–22 °C). Single cells lacking neurite-like processes were used, generally 2–5 days following plating on collagen-coated cover-slips in 35 mm culture dishes.

## Materials

[$^3$H]Noradrenaline ([$^3$H]NA; 16 Ci/mmol), $^{45}$CaCl$_2$ (35·12 mCi/mg), and $^{22}$NaCl (32 mCi/mg) were purchased from Amersham (Les Ulis, France). Pertussis toxin was from List Biologicals (Campbell, CA, USA); GTP, GDP-$\beta$-S, cholera toxin and tetrodotoxin were supplied by Calbiochem (La Jolla, CA, USA). Veratridine, apomorphine, acetylcholine and forskolin were from Sigma Chemical Co. (St Louis, MO, USA). Nicotine and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) were from Fluka Chemie AG (Buchs, Switzerland). LY 17 1555 was a gift from Eli Lilly (Fegersheim, France). Radioactivity was measured in scintillation vials containing 5 ml of Biofluor (New England, Dreieich, FRG) in a Packard scintillation counter. All other drugs and chemicals were of the best quality commercially available.

## Presentation of the data

Secretion and uptake experiments were repeated 2–5 times using different cell preparations. In each cell preparation, data are given as the mean of triplicate determinations on the same cell preparation $\pm$ S.E.M. In the figures, error bars smaller than the points are not indicated. In the electrophysiological experiments, data are presented as mean $\pm$ S.E.M. for $n$ cells, where appropriate.

<div align="center">RESULTS</div>

*Effects of apomorphine on catecholamine release and $^{45}Ca^{2+}$ uptake evoked by nicotine and 59 mM-$K^+$*

As illustrated in Fig. 1, the dopamine receptor agonist apomorphine caused a dose-dependent decrease in catecholamine release evoked by 20 $\mu$M-nicotine with an IC$_{50}$ of about 6 $\mu$M. At 100 $\mu$M, apomorphine decreased the net nicotine-stimulated catecholamine release to 16 % of the net nicotine-stimulated release obtained from

control cells. In contrast, chromaffin cells stimulated with 59 mM-K$^+$ are less sensitive to apomorphine, since at 100 $\mu$M-apomorphine, catecholamine release was still 64% of the net K$^+$-stimulated secretion from control cells (Fig. 1$A$). Apomorphine at concentrations up to 100 $\mu$M had no detectable effect on basal
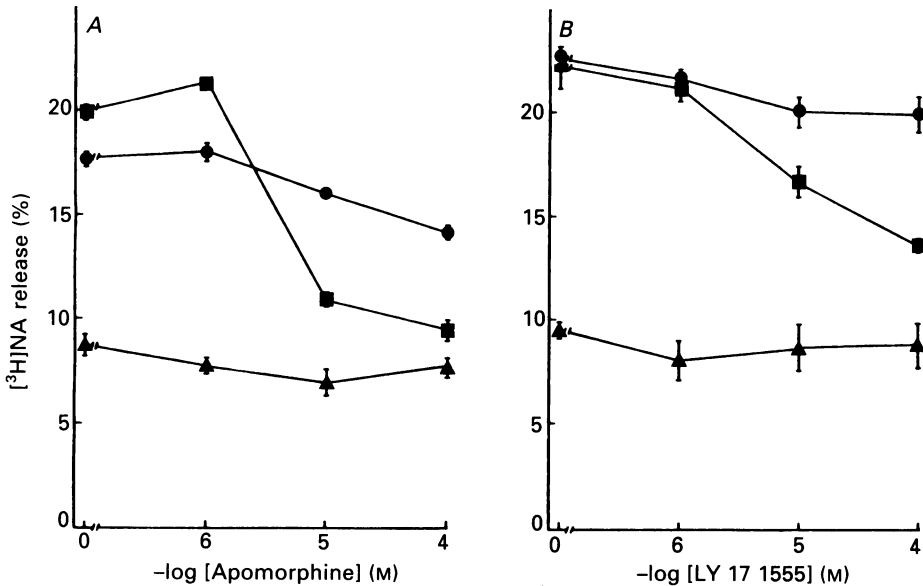


Fig. 1. Effect of apomorphine ($A$) and LY 17 1555 ($B$) on catecholamine secretion. Bovine adrenal chromaffin cells, pre-loaded with [$^3$H]NA, were incubated for 10 min in Locke solution containing various concentrations of apomorphine or LY 17 1555. Cells were then stimulated for 10 min with 20 $\mu$M-nicotine (■) or 59 mM-K$^+$ (●), in the presence of the indicated concentrations of apomorphine ($A$) or LY 17 1555 ($B$). Basal values (▲) were obtained by incubating the cells in Locke solution containing various concentrations of the D$_2$ agonists. [$^3$H]NA release was expressed as the percentage of the total radioactivity present in the cells prior to stimulation.

catecholamine secretion from resting chromaffin cells. Similar results were obtained using LY 17 1555, another specific D$_2$ agonist, as presented in Fig. 1$B$.

Depolarizing agents like KCl and veratridine stimulate Ca$^{2+}$ uptake and catecholamine secretion and Ca$^{2+}$ channel antagonists abolish these processes (Aguirre, Pinto & Trifaro, 1977; Schneider, Cline, Rosenheck & Sonenberg, 1981; Holz, Senter & Frye. 1982; Cena, Nicolas, Sanchez-Garcia, Kirpekar & Garcia, 1983; Artalejo *et al.* 1987; Kirshner, 1987), indicating a key role for voltage-sensitive Ca$^{2+}$ channels during secretion from chromaffin cells. Thus, a potential target for the inhibitory action of apomorphine on catecholamine release may be voltage-dependent Ca$^{2+}$ channels. As shown in Fig. 2, apomorphine produced a dose-dependent inhibition of nicotine-evoked $^{45}$Ca$^{2+}$ entry, with an IC$_{50}$ of approximately 5 $\mu$M, and at 100 $\mu$M apomorphine decreased the nicotine-evoked $^{45}$Ca$^{2+}$ entry to 15% of the net nicotine-stimulated control. In contrast, the high-K$^+$-evoked $^{45}$Ca$^{2+}$ uptake was less affected, being only reduced to 80% of the net high-K$^+$-stimulated control in the presence of 100 $\mu$M-apomorphine. Since depolarization with 59 mM-K$^+$ allows

a direct estimation of the $Ca^{2+}$ taken up through voltage-dependent channels, bypassing the cholinergic receptor, the inhibitory effect of apomorphine on $K^+$-evoked catecholamine release could be due to a direct inhibition of voltage-gated $Ca^{2+}$ channels (Bigornia *et al.* 1988). This possibility was directly tested by measuring the effect of apomorphine on $Ca^{2+}$ currents.
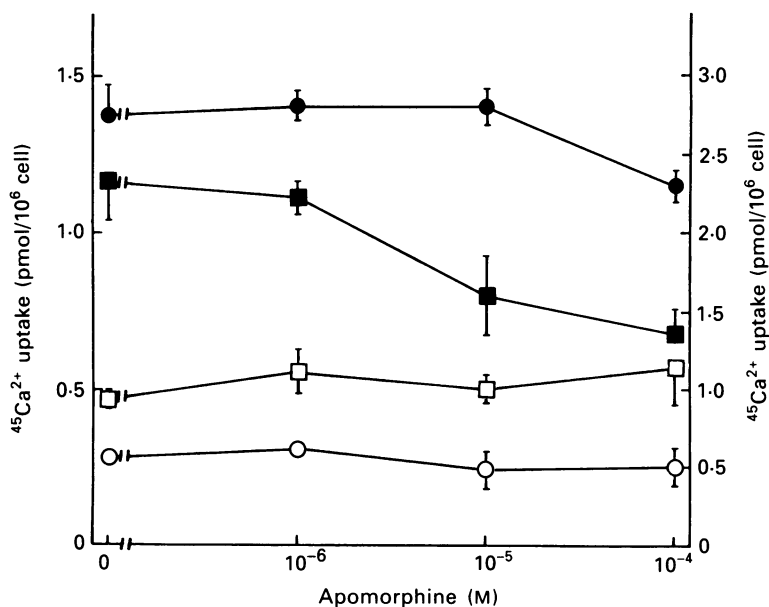


Fig. 2. Effect of apomorphine on secretagogue-evoked $^{45}Ca^{2+}$ uptake. Cells were pre-incubated for 10 min in Locke solution containing the indicated concentrations of apomorphine, followed by a 30 s stimulation with Locke solution (basal values, □, ○) or Locke solution containing either 20 μM-nicotine (■) or 59 mM-$K^+$ (●), in the presence of 1 μCi/0·2 ml $^{45}Ca^{2+}$ and apomorphine. Results were expressed as pmol of $^{45}Ca^{2+}$ taken up by $10^6$ cells/well. Left and right ordinates: $Ca^{2+}$ uptake in cells stimulated with nicotine and 59 mM-$K^+$ respectively. Apomorphine affected the secretagogue-induced $^{45}Ca^{2+}$ uptake in a similar manner compared to catecholamine secretion.

## Effect of apomorphine on voltage-dependent calcium current

In whole cell-recordings of $Ca^{2+}$ current from single chromaffin cells, application of 50 μM-apomorphine caused a reversible decrease in amplitude, as shown in Fig. 3A–C. In six cells, the maximal decrease amounted to $52 \pm 15 \%$ for apomorphine applications of 1–2 min. Both the onset of inhibition and recovery were fairly rapid, with a typical time course for the apomorphine-dependent action on normalized $Ca^{2+}$ current being illustrated in Fig. 3D. In some cells, more than one inhibitory response to apomorphine was obtained, and while the second apomorphine application produced a larger inhibition of $Ca^{2+}$ current for the cell shown in Fig. 3D, the second response in three other cells was not greatly different from the first. When quasi-steady-state current–voltage relations were obtained using fast voltage ramp commands (Fig. 3E), no voltage-dependent blocking actions of apomorphine were apparent.

Thus, dopamine agonist-mediated inhibition of depolarization-induced $Ca^{2+}$ influx and catecholamine release might be explained solely by inhibition of voltage-dependent $Ca^{2+}$ currents. However, an inhibitory effect at a step prior to $Ca^{2+}$ entry through voltage-gated channels can also be envisaged, since apomorphine has a more



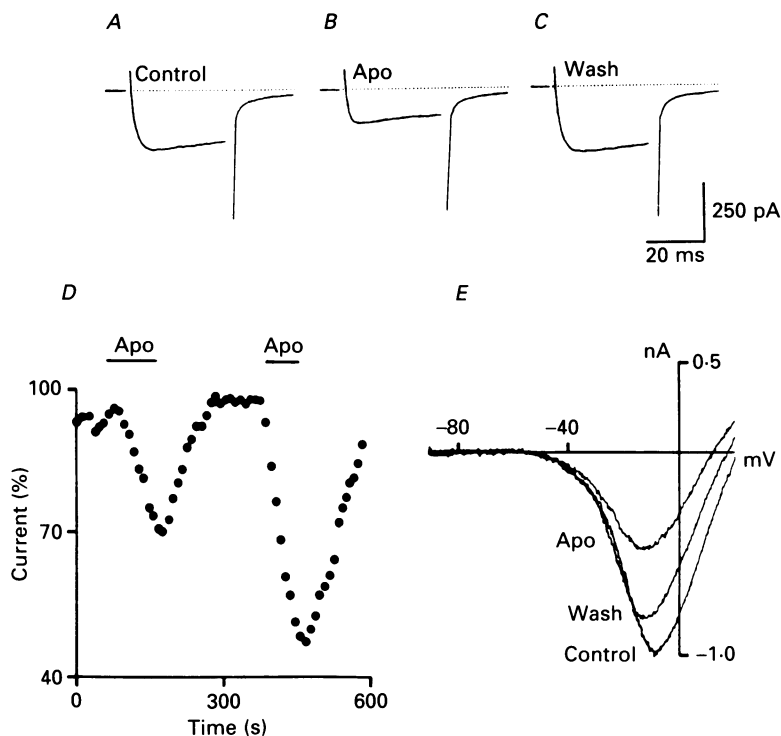Fig. 3. Whole-cell $Ca^{2+}$ currents are decreased by apomorphine. *A–C*, $Ca^{2+}$ currents elicited by 35 ms long voltage steps to 0 mV, from a holding potential of −90 mV, before (*A*, control), 1 min after continuous puffer application of 50 $\mu$M-apomorphine (*B*), and during recovery, 2 min after agonist application was stopped (*C*, wash). *D*, time course of action of apomorphine inhibition of normalized $Ca^{2+}$ current. Note that the ordinate scale is 40–100%. *E*, superimposed, quasi-steady-state, current–voltage relations for $Ca^{2+}$ current before (control), during 50 $\mu$M-apomorphine, and after recovery (wash), obtained using voltage ramp commands 150 ms in duration. *A–C*, *D* and *E* from separate cells. Note that the internal $Cs^{+}$ solution contained ATP, but no GTP. Filter, 1 kHz.

drastic effect on both $Ca^{2+}$ influx and catecholamine secretion when evoked by nicotine.

## Effect of apomorphine on $^{22}Na^{+}$ uptake

The main difference between nicotine- and high-$K^{+}$-induced secretion resides in the activation pathway for $Ca^{2+}$ entry (Kilpatrick, Slepetis & Kirshner, 1981*a*; Amy & Kirshner, 1982; Cena *et al.* 1983; Wada *et al.* 1984; Artalejo *et al.* 1987; Kirshner, 1987). Nicotine binds to its receptor and opens the associated ionophore, allowing mainly $Na^{+}$ entry (Fenwick, Marty & Neher, 1982*a*; Kidokoro, 1985) and local depolarization. This will then activate adjacent voltage-dependent $Na^{+}$ and $Ca^{2+}$ channels (Fenwick, Marty & Neher, 1982*b*). High-$K^{+}$ depolarization causes direct

opening of both voltage-sensitive $Na^+$ and $Ca^{2+}$ channels. Thus, while $Na^+$ entry is a limiting step for nicotine-stimulated $Ca^{2+}$ uptake and catecholamine release, it appears not to be essential in high-$K^+$-depolarized cells. Consequently, an alternative modulatory step for dopaminergic agonists in nicotine-stimulated catecholamine


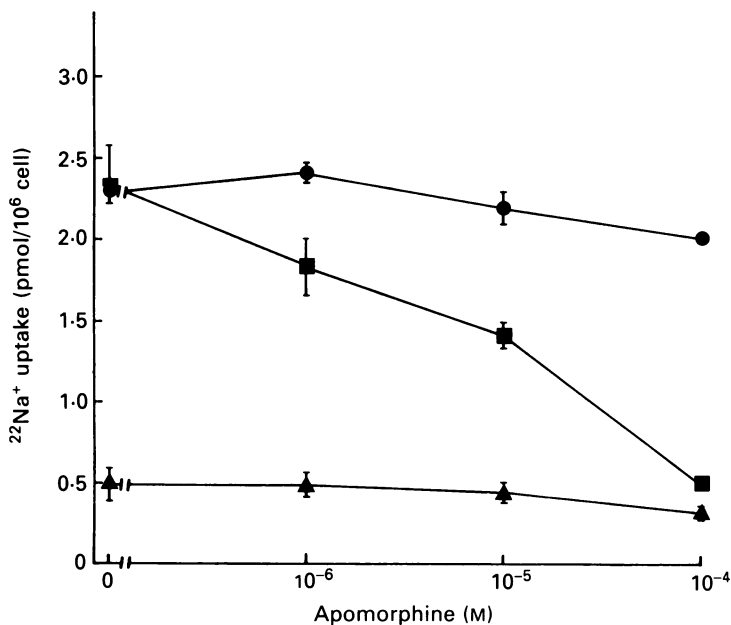
Fig. 4. Effect of apomorphine on secretagogue-evoked $^{22}Na^+$ uptake. Cultured chromaffin cells were treated for 10 min with increasing concentrations of apomorphine. Cells were subsequently stimulated for 5 min with Locke solution (basal release, ▲) or Locke solution containing either 20 $\mu$M-nicotine (■) or 59 mM-$K^+$ (●) in the presence of 1 $\mu$Ci/0·2 ml $^{22}Na^+$ and apomorphine.

release could be $Na^+$ entry via nicotinic receptors. This possibility was addressed in the experiment described in Fig. 4. The effect of various concentrations of apomorphine on $^{22}Na^+$ uptake was examined in cells stimulated for 10 min with either 20 $\mu$M-nicotine or 59 mM-$K^+$. Apomorphine caused a clear dose-dependent inhibition of the nicotine-evoked $^{22}Na^+$ uptake: with 100 $\mu$M-apomorphine, uptake was close to basal levels in resting cells, being only 9 % of the net nicotine-stimulated, control uptake. In contrast, $Na^+$ entry induced by 59 mM-$K^+$ was almost unaffected by apomorphine: $^{22}Na^+$ uptake in cells treated with 100 $\mu$M-apomorphine was 95 % of the net uptake measured in control cells.

Because $Na^+$ can enter chromaffin cells through either voltage-gated $Na^+$ channels or the ion channels associated with the nicotinic receptors (Kilpatrick et al. 1981 a, b; Amy & Kirshner, 1982; Wada et al. 1984), experiments with TTX, a specific blocker of voltage-dependent $Na^+$ channels, were designed to determine the $Na^+$ entry pathway modulated by apomorphine. In agreement with previous reports (Kilpatrick et al. 1981 a; Amy & Kirshner, 1982; Cena et al. 1983; Wada et al. 1984), TTX had no effect on either nicotine-evoked $^{22}Na^+$ uptake or catecholamine release (Table 1), indicating that $Na^+$ entry via voltage-gated $Na^+$ channels is negligible for these
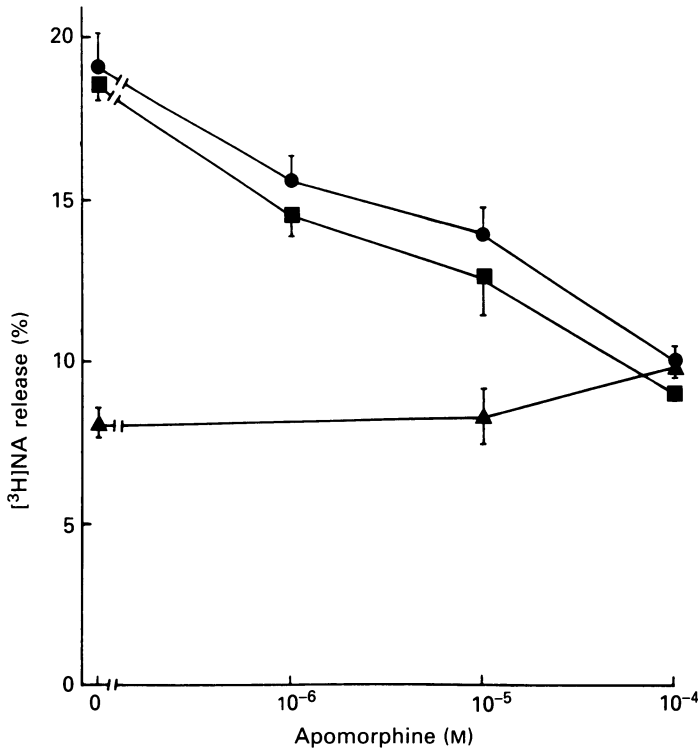
Fig. 5. Effect of apomorphine on catecholamine release evoked by two concentrations of the nicotinic agonist DMPP. Chromaffin cells pre-treated with the indicated concentrations of apomorphine were stimulated for 10 min with Locke solution in the absence (basal release, ▲) or presence of 10 $\mu$M (●) or 100 $\mu$M (■) DMPP. The dose–response curves to apomorphine were the same for the two concentrations of DMPP tested.

stimulation conditions. Apomorphine decreased both nicotine-induced $^{22}Na^+$ uptake and catecholamine release and TTX did not significantly modify these inhibitory effects (Table 1). This suggests that apomorphine may directly decrease the activity of the nicotinic receptor–ionophore complex.

We tested the possibility that apomorphine may compete with nicotine binding to its receptor by using chromaffin cells stimulated with two concentrations of the

TABLE 1. Effect of TTX on nicotine-evoked catecholamine secretion and $^{22}Na^+$ uptake

| | Control | Apomorphine | TTX | Apomorphine + TTX |
|---|---|---|---|---|
| | | [$^3$H]Noradrenaline release (%) | | |
| Locke | 7·3 | 7·0 | 8·8 | 6·7 |
| Nicotine | 24·9 ± 0·31 | 9·6 ± 0·61 | 24·5 ± 0·30 | 7·6 ± 0·52 |
| | | $^{22}Na^+$ uptake (pmol/10$^6$ cells) | | |
| Locke | 0·85 ± 0·07 | 0·75 ± 0·04 | 0·88 ± 0·08 | 0·55 ± 0·01 |
| Nicotine | 2·08 ± 0·14 | 0·94 ± 0·30 | 2·12 ± 0·05 | 0·91 ± 0·07 |

Cultured chromaffin cells were incubated in Locke solution in the presence or absence of 100 $\mu$M-apomorphine. Cells were then stimulated with Locke solution (Locke) or Locke solution containing 20 $\mu$M-nicotine (nicotine), in the absence (control) or presence of 100 $\mu$M-apomorphine and/or 10 $\mu$M-TTX.

nicotinic agonist DMPP. As illustrated in Fig. 5, apomorphine caused a similar, dose-dependent inhibition of catecholamine release for cells stimulated with either 10 or 100 $\mu$M-DMPP; $IC_{50}$ values for apomorphine were 5 and 7 $\mu$M, respectively. Thus, apomorphine-induced inhibition of secretion is not competitive with DMPP (Fig. 5).
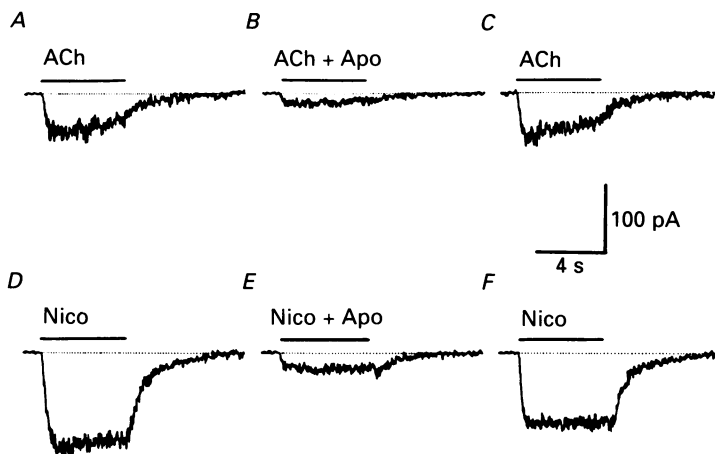


Fig. 6. Effect of apomorphine on ACh- and nicotine-evoked inward currents recorded under whole-cell voltage clamp. *A–C*, inward currents produced by 50 $\mu$M-ACh before (*A*, control), during (*B*, test) and after (*C*, recovery) simultaneous application of 50 $\mu$M-apomorphine in a single chromaffin cell. *D–F*, inward currents produced by 10 $\mu$M-nicotine before (*D*), during (*E*) and after (*F*) simultaneous application of 5 $\mu$M-apomorphine in another cell. Bars indicate times of application of the agonists using puffer pipettes, in the absence or presence of apomorphine. Holding potential, $-90$ mV. Filter, 800 Hz.

Similar results were obtained with nicotine (not shown). These data suggest that apomorphine is not acting at the nicotinic receptor binding site.

*Effect of apomorphine on nicotinic receptor-mediated ion currents*

Inward current evoked by acetylcholine (ACh) and nicotine were recorded under whole-cell voltage clamp (Fig. 6). Puffer pipette distance from the cell and applied pressure were adjusted so that maintained, inward currents were observed, using a $-90$ mV holding potential. In some cells, 50 $\mu$M-apomorphine almost completely blocked nicotinic current produced by 50 $\mu$M-ACh (Fig. 6*A–C*). Note that the blocking action was rapid, with current amplitudes being reduced as soon as nicotinic current was apparent. The block was reversible, with recovery to control levels within 3 min of wash-out. In fourteen cells, 50 $\mu$M-apomorphine reduced the inward current evoked by 50 $\mu$M-ACh to $31 \pm 4$% of the control values observed with ACh alone. A similar, reversible, almost complete inhibition by apomorphine (5 $\mu$M) was observed when 10 $\mu$M-nicotine was the applied agonist, as shown for another cell in Fig. 6*D–F*. In ten cells, 5 $\mu$M-apomorphine reduced inward current induced by 10 $\mu$M-nicotine to $28 \pm 5$% of the control values, without apomorphine. Note that more than one inhibitory response to apomorphine could be obtained from the majority of the cells tested ($n = 12$); trials were spaced at intervals of about 10 min. These subsequent responses were not apparently different in amplitude or time course from

the first observed, suggesting that diffusible second messengers which are often diluted out in the whole-cell recording mode are probably not important for the blocking action of apomorphine on nicotinic currents.

*Lack of effect of apomorphine on voltage-gated sodium channels*

The effects of apomorphine and TTX were examined on catecholamine release evoked by veratridine, which is known to trigger secretion via activation of voltage-dependent $Na^+$ channels. As shown in Table 2, TTX (10 $\mu$M) completely blocked the veratridine-evoked catecholamine release, whereas the maximal concentration of

TABLE 2. Effect of TTX on veratridine- and high-$K^+$-induced catecholamine secretion

[$^3$H]Noradrenaline release (%)

| | Control | Apomorphine | TTX | Apomorphine + TTX |
|---|---|---|---|---|
| Locke | 8·1 ± 0·3 | 8·2 ± 0·7 | 8·8 ± 0·2 | 9·8 ± 0·3 |
| Veratridine | 23·4 ± 0·5 | 16·6 ± 0·3 | 8·1 ± 0·2 | 7·3 ± 0·3 |
| High $K^+$ | 22·8 ± 0·5 | 16·2 ± 0·4 | 19·1 ± 0·3 | 13·0 ± 0·6 |

Chromaffin cells were incubated for 10 min in the presence or absence of 100 $\mu$M-apomorphine. Cells were subsequently stimulated with Locke solution alone, or Locke solution containing either 50 $\mu$M-veratridine or 59 m$M$-$K^+$. Stimulation was performed in the presence or absence of 100 $\mu$M-apomorphine and/or 10 $\mu$M-TTX.

apomorphine (100 $\mu$M) caused only a partial inhibition. TTX caused a 16% inhibition of the catecholamine release induced by 59 m$M$-$K^+$, and apomorphine (100 $\mu$M) caused a 29% inhibition of high-$K^+$-stimulated catecholamine release, while apomorphine together with TTX resulted in 43% inhibition of $K^+$-evoked release (Table 2). The inhibitory effects of apomorphine and TTX on secretion induced by 59 m$M$-$K^+$ appear to be additive, indicating that the sites of action for apomorphine and TTX are different.

Whole-cell recordings of voltage-activated $Na^+$ currents failed to reveal any effect of apomorphine (50 $\mu$M) on peak amplitude (Fig. 7$A$–$C$), or on quasi-steady-state current–voltage relations (Fig. 7$D$). Similar results were obtained from a total of six cells. Together, these observations indicate that the inhibitory action of apomorphine is not likely to involve TTX-sensitive $Na^+$ channels.

*Effect of apomorphine on potassium conductance*

The effects of apomorphine on nicotine-induced $Ca^{2+}$ influx and catecholamine release described above might be first mediated by an increase in $K^+$ conductance, which could lead to membrane hyperpolarization and a subsequent decrease in the probability of opening of $Ca^{2+}$ channels. When whole-cell $K^+$ currents were elicited by voltage-steps from $-90$ mV to a test potential of 40 mV, a large, reversible decrease total outward current was observed in the presence of 100 $\mu$M-apomorphine (Fig. 8$A$). However, because most of total outward $K^+$ current in chromaffin cells results from $Ca^{2+}$-activated $K^+$ channels (Marty & Neher, 1985), it may be that the observed decrease in outward current is secondary to the apomorphine-dependent decrease in inward $Ca^{2+}$ current described in Fig. 3. Consequently, the effect of apomorphine on $K^+$ currents was tested after $Ca^{2+}$ currents were first blocked by

adding 1 mM-CdCl$_2$ to the bath solution. As illustrated in Fig. 8*B* for another cell, addition of 1 mM-CdCl$_2$ resulted in a large decrease in outward current, in agreement with previous work (Marty & Neher, 1985). Subsequent exposure to 100 $\mu$M-apomorphine again gave rise to a reversible decrease in outward K$^+$ current (Fig. 8*B*), although the decrease was smaller than in Fig. 8*A*.
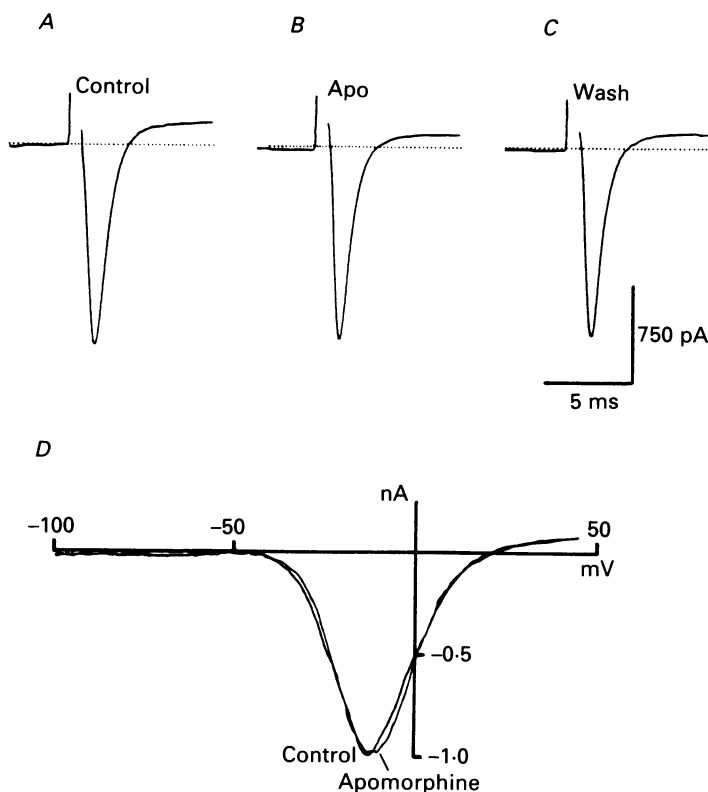


Fig. 7. Apomorphine does not affect voltage-activated sodium currents. *A–C*, sodium currents (control and wash) elicited by voltage clamp steps from $-90$ to $-10$ mV were unaffected by 50 $\mu$M-apomorphine (Apo). *D*, in another cell, quasi-steady-state current–voltage relations obtained with a fast voltage ramp command (12 ms long, from $-100$ to 50 mV) were unchanged by 50 $\mu$M-apomorphine, compared to control. Filter, 3 kHz.

This protocol does not, however, allow demonstration of a possible apomorphine-dependent increase in resting K$^+$ conductance, as the holding potential of $-90$ mV is very close to $-85$ mV, the expected K$^+$ equilibrium potential with our solutions. To address this possibility, voltage ramp commands 200 ms in duration were given from $-110$ to 80 mV, as illustrated in Fig. 8*C*. No change in the quasi-steady-state current–voltage relations in the voltage range of $-110$ to $-40$ mV was apparent in the presence of 100 $\mu$M-apomorphine. This indicates clearly the lack of putative apomorphine-dependent increases in K$^+$ conductance at potentials close to the normal cell resting potential. Apomorphine again caused a decrease in peak total outward K$^+$ current, although much smaller than in Fig. 8*A*. Further, under whole-

cell current clamp with or without 200 $\mu$M-GTP in the pipette internal solution, apomorphine application did not lead to hyperpolarization of the normal resting potential, which was about $-50$ to $-60$ mV (not shown; $n = 3$). These results clearly demonstrate that the inhibitory effect of apomorphine on secretagogue-evoked $Ca^{2+}$



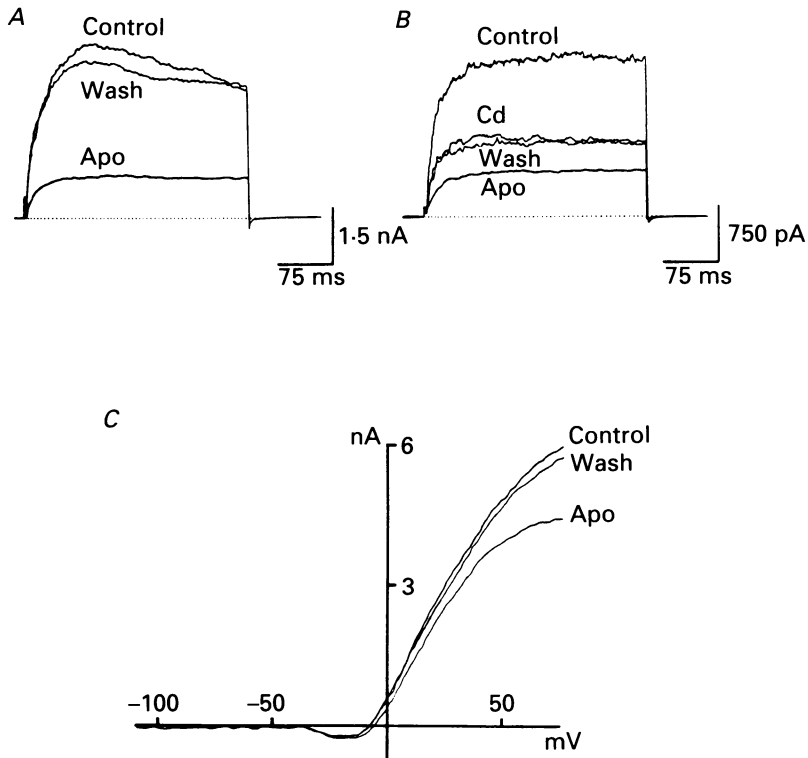Fig. 8. Effect of apomorphine on $K^+$ currents. *A*, superimposed current traces before (control), during 100 $\mu$M-apomorphine (Apo) and upon wash-out (wash) for voltage steps from $-90$ to 40 mV. *B*, bath addition of 1 mM-CdCl$_2$ (Cd) causes large decease on outward current (upper two traces); a reversible decrease in outward current due to apomorphine (100 $\mu$M) is seen (lower two traces). The puffer pipette also contained CdCl$_2$. Holding potential, $-90$ mV; test potential, 40 mV. *C*, quasi-steady-state current–voltage relations obtained with normal external $Na^+$ and internal $K^+$ solutions using 200 ms long, voltage ramp commands from $-110$ to 70 mV before (control), during 100 $\mu$M-apomorphine (Apo), and after recovery (wash). *A*, *B* and *C* from three separate cells. Filter, 1 kHz.

influx and catecholamine release cannot be explained by a mechanism involving increases in $K^+$ conductance and membrane hyperpolarization.

*Effect of apomorphine on action potentials*

In an effort to relate these different electrophysiological effects of apomorphine to secretion under conditions closer to those *in situ*, action potentials were recorded in whole-cell current clamp. Usually, small steady hyperpolarizing currents were applied to fix the resting potential around $-80$ mV. Action potentials were elicited
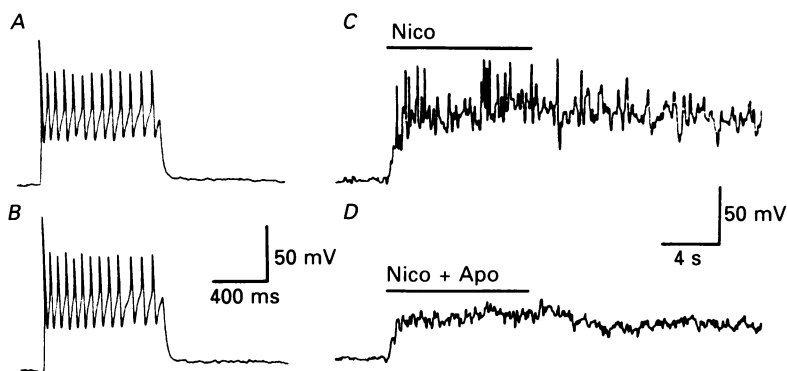
Fig. 9. The effect of apomorphine on action potentials elicited electrically or by nicotine application under whole-cell current clamp. A and B, action potential activity (A) induced by depolarizing current pulses is not modified in the presence of 5 $\mu$M-apomorphine (B). C and D, in another cell, action potentials evoked by 50 $\mu$M-nicotine (C) were blocked by 5 $\mu$M-apomorphine (D). Resting potentials initially were adjusted to $-80$ mV using small steady hyperpolarizing currents.

TABLE 3. Effects of pertussis and cholera toxins on apomorphine-dependent inhibition of nicotine and high-K$^+$-evoked catecholamine release

|  | Apomorphine-induced inhibition (%) | |
| --- | --- | --- |
|  | Nicotine | 59 mM-K$^+$ |
| Pertussis toxin (ng/ml) | | |
| 0 | $53 \cdot 3 \pm 4 \cdot 1$ | $53 \cdot 2 \pm 2 \cdot 3$ |
| 10 | $53 \cdot 3 \pm 4 \cdot 7$ | $44 \cdot 5 \pm 3 \cdot 8$ |
| 100 | $62 \cdot 1 \pm 5 \cdot 1$ | $58 \cdot 1 \pm 2 \cdot 1$ |
| 1000 | $59 \cdot 8 \pm 2 \cdot 1$ | $52 \cdot 4 \pm 2 \cdot 7$ |
| Cholera toxin (ng/ml) | | |
| 0 | $53 \cdot 7 \pm 7 \cdot 1$ | $53 \cdot 0 \pm 3 \cdot 6$ |
| 10 | $61 \cdot 8 \pm 3 \cdot 3$ | $51 \cdot 7 \pm 6 \cdot 7$ |
| 100 | $65 \cdot 7 \pm 3 \cdot 1$ | $44 \cdot 8 \pm 4 \cdot 2$ |
| 1000 | $64 \cdot 5 \pm 3 \cdot 7$ | $55 \cdot 1 \pm 3 \cdot 4$ |

Cultured chromaffin cells were pre-loaded with [$^3$H]NA and then incubated for 4 h with the indicated concentrations of either pertussis or cholera toxins in culture medium. Cells were subsequently washed and incubated for 10 min in the presence or absence of 10 or 100 $\mu$M-apomorphine, for nicotine-stimulated or K$^+$-stimulated cells, respectively. Cells were then stimulated for 10 min with 20 $\mu$M-nicotine or 59 mM-K$^+$ in the presence or absence of apomorphine. Calculation of percentage inhibition was for net secretory values, obtained after subtraction of basal values. Absolute secretory values in the absence of apomorphine were $19 \cdot 6 \pm 1 \cdot 2$ and $23 \cdot 7 \pm 0 \cdot 3$ % of total catecholamine content, for nicotine and K$^+$ stimulation, respectively. Basal release was $9 \cdot 2 \pm 0 \cdot 3$ % of total content, and was not significantly modified over the range of toxin concentrations used.

either directly by passing depolarizing current pulses or by nicotine application, in the absence or presence of apomorphine. Upon just supramaximal electrical stimulation, a rapid depolarization resulting in maintained action potential activity was observed (Fig. 9A), similar to K$^+$-evoked spiking described earlier (Kidokoro & Ritchie, 1980). When this was repeated in the presence of 5 $\mu$M-apomorphine, no

obvious changes in action potential behaviour were apparent (Fig. 9*B*), as was the case for four other cells. Action potentials arising after depolarization induced by 50 $\mu$M-nicotine were less regular in frequency (Fig. 9*C*), although this was variable from cell to cell (Kidokoro, Miyazaki & Ozawa, 1982). When the cell was then
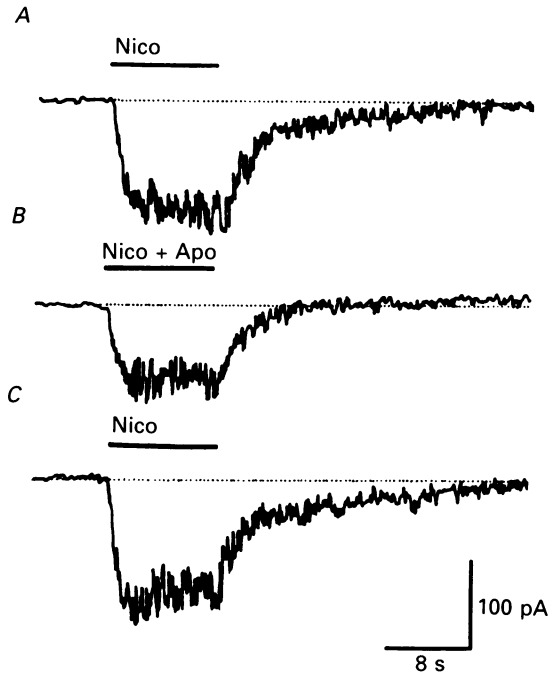


Fig. 10. Apomorphine inhibition of currents activated by 10 $\mu$M-nicotine are unaffected by inclusion of 200 $\mu$M-GDP-$\beta$-S in the pipette internal solution. *A–C*, inward whole-cell currents before (*A*), in the presence of 5 $\mu$M-apomorphine (*B*), and after wash-out (*C*), from a single chromaffin cell. Holding potential, $-90$ mV. Filter, 800 Hz.

stimulated with 50 $\mu$M-nicotine together with 5 $\mu$M-apomorphine (Fig. 9*D*), no action potentials were elicited, and the resulting cell depolarization was smaller than in the absence of apomorphine. Complete block or drastic reduction of nicotine-stimulated spiking by apomorphine was observed in six cells, somewhat reminiscent of the action of dopamine on spiking in lactotrophs (Douglas & Taraskevich, 1982).

*Role of G-proteins in apomorphine action*

The responses elicited by the activation of various types of inhibitory receptors, including those for somatostatin in GH$_3$ cells (Koch, Dorflinger & Schönbrunn, 1985) and for dopamine in lactotroph cells (Malgaroli, Vallar, Elahi, Pozzan, Spada & Meldolesi, 1987), have been reported to be blocked by pre-treatment of the cells with pertussis toxin (PTX). In chromaffin cells, treatment with various concentrations of PTX (up to 1 $\mu$g/ml for 4 h) did not affect the apomorphine-induced inhibition of nicotine-dependent catecholamine release (Table 3). Similarly, PTX

did not modify the inhibitory effect of apomorphine on nicotine-stimulated $^{22}Na^+$ influx (not shown). These data suggest that the mechanism by which apomorphine induces its inhibitory effect on nicotine-stimulated responses does not involve a G-protein sensitive to PTX.
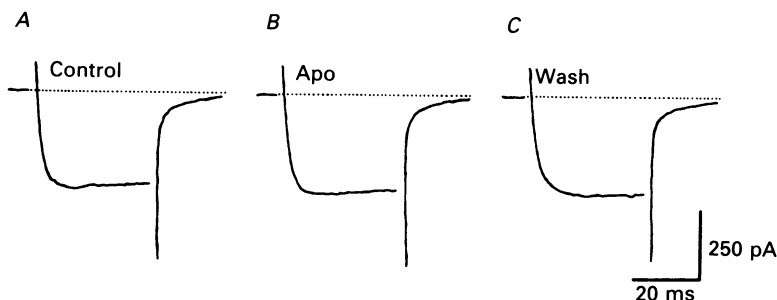


Fig. 11. Apomorphine-induced decreases in $Ca^{2+}$ current are blocked when 200 $\mu$M-GDP-$\beta$-S is included in the pipette internal solution. *A*, control $Ca^{2+}$ current. *B*, in the presence of 50 $\mu$M-apomorphine. *C*, after wash-out. Holding potential, $-90$ mV; test pulses to 0 mV. Filter, 1 kHz.

Another way to affect G-protein function is to use GDP-$\beta$-S, a non-hydrolysable GDP analogue. As illustrated in Fig. 10, the apomorphine-dependent decrease in inward whole-cell current activated by nicotine was still observed when 200 $\mu$M-GDP-$\beta$-S was included in the pipette internal solution. Cells were dialysed in the whole-cell recording mode for at least 5 min before responses were acquired for analysis. Similar results were obtained in eight cells, with 5 $\mu$M-apomorphine reducing the inward current activated by 10 $\mu$M-nicotine to $40\pm8$% of control levels observed with nicotine alone. Note also that apomorphine-induced decreases in nicotinic current and $Ca^{2+}$ current (described above) were observed in the absence of GTP in the pipette internal solutions; inclusion of 200 $\mu$M-GTP did not apparently modify the effects of apomorphine (not shown).

However, when apomorphine (50 $\mu$M) was tested on voltage-activated, whole-cell $Ca^{2+}$ currents with 200 $\mu$M-GDP-$\beta$-S included in the pipette internal solution (Fig. 11), the inhibition of $Ca^{2+}$ current described above in Fig. 3 was no longer observed. Similar results were obtained in a total of seven cells and are in agreement with the G-protein-coupled dopaminergic inhibition of $Ca^{2+}$ current described in snail neurones (Harris-Warrick, Hammond, Paupardin-Tritsch, Homburger, Rouot, Bockaert & Gerschenfeld, 1988). However, PTX did not modify the apomorphine-dependent inhibition of high-$K^+$-evoked catecholamine release (Table 3). These data indicate that the G-protein involved in coupling $D_2$ receptor activation to decreases in voltage-dependent $Ca^{2+}$ current in chromaffin cells is not PTX sensitive.

*Elevation of cyclic AMP does not modify the effects of apomorphine*

A well-known effect of dopamine receptor activation, for example in pituitary lactotrophs, is inhibition of adenylate cyclase, resulting in a marked decrease of cyclic AMP levels (De Camilli & Macconi, 1979; Swennen & Denef, 1982; Delbeke & Dannies, 1985; Enjalbert, Sladeczek, Guillon, Bertrand, Shu, Epelbaum, Garcia-

Sainz, Jard, Lombard, Kordon & Bockaert, 1986). In order to establish whether the effects of apomorphine on ionic currents and catecholamine release might involve a cyclic AMP-dependent mechanism, treatments that cause elevation of cyclic AMP were given to chromaffin cells prior to exposure to apomorphine and stimulation with nicotine or 59 mM-$K^+$. The mean cyclic AMP level in cultured chromaffin cells was $0.32 \pm 0.1$ pmol/$10^6$ cells. Treatment with cholera toxin (up to 1 $\mu$g/ml for 4 h) or forskolin (10 $\mu$M) produced a clear elevation of intracellular cyclic AMP levels to respectively $5.44 \pm 0.96$ and $4.10 \pm 0.59$ pmol/$10^6$ cells. However, pre-treatment of cells with CTX (Table 3) or forskolin (not shown) left the inhibition produced by 10 $\mu$M-apomorphine on nicotine-evoked catecholamine release and $^{22}Na^+$ uptake (not shown) entirely unaffected. Similarly, CTX did not modify the inhibitory effects of 100 $\mu$M-apomorphine on high-$K^+$-evoked catecholamine secretion (Table 3).

## DISCUSSION

The major findings reported here are that dopamine agonist-dependent decreases in catecholamine secretion are associated with decreases in current through voltage-sensitive $Ca^{2+}$ channels and also through nicotinic receptor/channels. Somewhat surprisingly, no evidence was found for an apomorphine-dependent increase in $K^+$ conductance.

The presence of inhibitory dopamine receptors in a wide variety of tissues in the peripheral nervous system (Long, Heintz, Cannon & Kim, 1975; Hope, McCulloch, Storey & Rand, 1977; Steinsland & Hieble, 1978), including the adrenal medulla, is well established. The reversal of the inhibitory effects of dopamine agonists on catecholamine release by specific $D_2$ antagonists such as sulpiride indicates that the $D_2$ receptor subtype is responsible, both in the adrenal gland (Artalejo *et al.* 1985; Gonzalez *et al.* 1986) and in cultured chromaffin cells (Bigornia *et al.* 1988). To date, there is no evidence for $D_1$ receptors in the adrenal medulla and $D_1$ agonists do not inhibit catecholamine release nor do $D_1$ antagonists prevent the inhibitory effect of apomorphine (Bigornia *et al.* 1988).

Our objective was to elucidate the mechanisms by which dopaminergic agonists inhibit catecholamine release from adrenal chromaffin cells. Based on the apomorphine-induced block of both nicotine- and high-$K^+$-evoked $Ca^{2+}$ influx and catecholamine release, dopamine receptors on chromaffin cells were suggested to alter $Ca^{2+}$ channel conductance (Bigornia *et al.* 1988). Our results are not in complete agreement with this study, as the effects of apomorphine depended on the secretagogue used to stimulate the cells. Nicotine-stimulated $Ca^{2+}$ influx and catecholamine release were greatly decreased by apomorphine and LY 17 1555, but only partial inhibition of these processes was observed with apomorphine and LY 17 1555 when they were evoked by 59 mM-$K^+$.

In bovine chromaffin cells, it is well known that cholinergic agonists induce opening of ion channels associated with nicotinic receptors, giving rise to $Na^+$ influx (Kilpatrick *et al.* 1981*b*; Fenwick *et al.* 1982*a*; Amy & Kirshner, 1982; Cena *et al.* 1983; Corcoran & Kirshner, 1983; Wada *et al.* 1984), depolarization and the firing of $Ca^{2+}$ and $Na^+$-dependent action potentials (Kidokoro, 1985). $Na^+$ entry through voltage-dependent channels plays a facilitatory but not obligatory role in stimulation

of secretion by cholinergic agonists and is observed only when stimulation is induced by low levels of agonists (Kidokoro, 1985; Kirshner, 1987). In contrast, high-$K^+$ depolarization directly opens both voltage-sensitive $Na^+$ and $Ca^{2+}$ channels, and thus, the main difference between nicotine- and high-$K^+$-evoked responses is the requirement for $Na^+$ entry with nicotinic stimulation (Cena *et al.* 1983). While we have directly demonstrated an apomorphine-induced decrease of voltage-sensitive $Ca^{2+}$ current similar to that found in chick (Marchetti, Carbone & Lux, 1986) and snail neurones (Harris-Warrick *et al.* 1988) and in rat melanotroph cells (Cota & Hiriart, 1989), the dependence of apomorphine inhibition of secretion and $Ca^{2+}$ uptake on the secretagogue used suggests that $Ca^{2+}$ channels are not solely affected. With regards to voltage-dependent $Na^+$ channels, TTX had no effect on $^{22}Na^+$ uptake either in control nicotine-stimulated cells or in apomorphine-treated cells. However, apomorphine completely abolished nicotine-evoked $^{22}Na^+$ uptake and secretion in a non-competitive manner and significantly reduced whole-cell inward currents activated by ACh and nicotine. These data demonstrate a clear inhibitory effect of apomorphine on nicotinic receptor/channel function. Nevertheless, the possibility that apomorphine directly modulates $Na^+$ entry via voltage-gated channels was examined. TTX completely blocked $Na^+$ influx and catecholamine secretion induced by veratridine. In contrast, apomorphine caused only a partial inhibition of the veratridine-stimulated responses. Moreover, whole-cell $Na^+$ currents activated by voltage steps were unaffected by 50 $\mu$M-apomorphine, and taking the data together, it is clear that apomorphine does not act on TTX-sensitive $Na^+$ channels.

A common dopamine-dependent action in different cell types has been described, where membrane hyperpolarization results from the activation of a $K^+$ conductance (Ingram, Bicknell & Mason, 1986; Israel, Kirk & Vincent, 1987; Lacey, Mercuri & North, 1987; Sasaki & Sato, 1987; Freedman & Weight, 1988; Castelletti, Memo, Missale, Spano & Valerio, 1989; Gregerson, Einhorn, Smith & Oxford, 1989). This membrane hyperpolarization might then secondarily lead to diminished $Ca^{2+}$ entry, and decreased secretion. However, two lines of evidence indicate that $D_2$ receptors in chromaffin cells do not induce increases in $K^+$ conductance. Firstly, no signs of outward current activation or membrane hyperpolarization around the normal resting potential were seen in whole-cell recordings. Indeed, an apomorphine-dependent decrease in total outward current was observed at depolarized potentials. Secondly, apomorphine did not change the frequency of electrically driven action potentials under current clamp. These results strongly suggest that the mechanism linking $D_2$ receptor activity to inhibition of catecholamine secretion in chromaffin cells does not involve the activation of $K^+$ channels and membrane hyperpolarization.

The inhibition of secretion mediated by $D_2$ receptor activation has been investigated in detail in pituitary lactotrophs (Ben-Jonathan, 1985; Vallar & Meldolesi, 1989). In these cells, the dopamine receptor seems to be coupled to at least two separate effectors, which result in a decrease in adenylate cyclase activity and activation of $K^+$ channels (and possibly inhibition of $Ca^{2+}$ channels). Using cyclic AMP-elevating agents, it was shown that the inhibition of $[Ca^{2+}]_i$ transients induced by $D_2$ activation is related to decreased adenylate cyclase activity and diminished cytosolic cyclic AMP levels (Malgaroli *et al.* 1987). However, in chromaffin cells, we

found that both cholera toxin and forskolin significantly enhanced cytosolic cyclic AMP but did not antagonize the inhibitory effects of apomorphine on catecholamine secretion, implying that decreases in cyclic AMP were not involved. In lactotrophs, pertussis toxin, which ADP-ribosylates members of the $G_i/G_o$ protein family, blocked the effect of $D_2$ receptors on adenylate cyclase and also inhibited the decreases in $[Ca^{2+}]_i$ and the hyperpolarization induced by dopamine (Vallar & Meldolesi, 1989). In contrast, we found that PTX at 1 $\mu$g/ml (a concentration which produces complete ADP-ribosylation in chromaffin cells; Bittner, Holz & Neubig, 1986) did not affect dopaminergic inhibition of nicotine- and high-$K^+$-evoked catecholamine secretion, implying that a PTX-sensitive G-protein was not involved. This however does not exclude the possibility that G-proteins insensitive to PTX might be implicated. Indeed, recovery experiments using $D_2$ receptors partially purified from bovine anterior pituitary (Senogles, Benovic, Amlaiky, Unson, Milligan, Vinitsky, Spiegel & Caron, 1987) and reconstruction of porcine striatal $D_2$ receptors with G-protein preparations (Ohara, Haga, Bernstein, Haga, Ichiyama & Ohara, 1988) suggest that at least two different G-proteins play a role in coupling $D_2$ receptors to their effectors. The block of apomorphine-induced inhibition of voltage-dependent $Ca^{2+}$ currents when GDP-$\beta$-S was included in the pipette internal solution supports this idea (see also Harris-Warrick *et al.* 1988). In contrast, the apomorphine modulation of nicotinic currents was still observed when GDP-$\beta$-S was present in the pipette internal solution, an observation arguing against a role for G-proteins at least in coupling $D_2$ receptors to nicotinic receptor/channels.

Apomorphine may inhibit current through nicotinic channels by an open channel blocking mechanism, as suggested both by the rapidity of the onset of block (Fig. 6) and by the small increase in current sometimes seen when apomorphine application was stopped (Fig. 6*E*). Similarly, $\alpha$-adrenergic agonists like clonidine cause decreases in nicotine-evoked catecholamine secretion in chromaffin cells, an effect ascribed to an open channel blocking action of clonidine on inward nicotinic currents (Cull-Candy, Mathie & Powis, 1988). A channel blocking action of atrial natriuretic factor on nicotinic currents in bovine chromaffin cells has also been recently described (Bormann, Flügge & Fuchs, 1989).

Ion channel activity has been found to be directly modulated by intracellular messengers like cyclic GMP, by G-proteins directly coupled to channels, and by protein kinases stimulated by internal messengers such as $Ca^{2+}$ or cyclic AMP (Rosenthal & Schultz, 1987). Dopamine interacts with the phosphatidylinositol/$Ca^{2+}$ mobilization pathway in lactotrophs (Canonico, Valdenegro & Macleod, 1982; Enjalbert *et al.* 1986) and consequently may modulate protein kinase C activity. Since in some cases, the activity of voltage-gated $Ca^{2+}$ channels and nicotinic ion channels appears to be regulated by protein kinase C-mediated phosphorylation (Levitan, 1985), it is tempting to speculate that the mechanism of action of dopaminergic modulation of adrenomedullary catecholamine secretion might also involve protein kinase C. Apomorphine inhibition was unaltered by PTX pre-treatment, although it is possible in chromaffin cells that PTX-insensitive pathways of phosphatidylinositol hydrolysis exist, as described in other systems (Ashkenazi, Peralta, Winslow, Ramachandran & Capon, 1989).

From a functional point of view, dopamine agonist-dependent decreases of

nicotine-evoked catecholamine release in perfused cat and bovine adrenal glands suggest an *in situ* negative feedback control by dopamine (Artalejo *et al.* 1985; Gonzales *et al.* 1986). The source of dopamine may be from co-stored dopamine (Menniti & Diliberto, 1989) released together with catecholamines from secretory granules, or alternatively, as in sympathetic ganglia, specific dopamine-containing cells may also be present in the adrenal medulla. The lack of apparent effect of apomorphine on electrically evoked action potentials may be misleading with respect to $Ca^{2+}$ entry necessary for secretion, as inward $Na^+$ current density is much greater than that for $Ca^{2+}$ (Fenwick *et al.* 1982*b*). Further, because secretion is normally triggered by pulsatile release of ACh, the respective concentration profiles of ACh and dopamine present *in situ* need to be documented. Thus, while the action potential data suggest that dopaminergic inhibition of nicotine-evoked activity would play a preponderant role in the inhibition of secretion, it is difficult to evaluate the relative importance *in situ* of the apomorphine-induced inhibition of $Ca^{2+}$ current compared to its effect on nicotinic current as examined here.

In summary, our work demonstrates that apomorphine inhibits catecholamine secretion from bovine adrenomedullary chromaffin cells by reducing $Ca^{2+}$ entry through voltage-gated $Ca^{2+}$ channels and also by decreasing current through nicotinic receptor-associated channels. Unlike other types of cells such as lactotrophs, chromaffin cells do not respond to $D_2$ receptor stimulation by increases of $K^+$ conductance. Future studies should further define the molecular mechanisms involved and their relative importance for the action of $D_2$ agonists on different types of ion channels in the dopaminergic inhibition of secretion from adrenomedullary chromaffin cells.

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