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# ELECTROPHYSIOLOGICAL RESPONSES TO DOPAMINE OF RAT HYPOPHYSIAL CELLS IN LACTOTROPH-ENRICHED PRIMARY CULTURES

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SUMMARY

1. Cells from 14-day-old and lactating female rat pituitary glands were dissociated, separated and enriched on a continuous gradient of bovine serum albumin at unit gravity. They were maintained for at least 6 days in culture before perifusion and electrophysiological experiments were performed.

2. Immunofluorescent staining of the resulting gradient fractions (numbered F2 to F9) from both groups of animals indicated that the majority of lactotrophs were located in the light fractions (F3–F4). However, a second population of lactotrophs was observed in the heavy fractions (F7–F9) isolated from lactating females.

3. Basal secretion rates of prolactin were in the order of 2–40 ng 2 $min^{-1}$ $10^6$ $cells^{-1}$ and were inhibited by dopamine in a dose-dependent manner.

4. According to their electrophysiological properties, cells from 14-day-old females (first group) were categorized as follows: (1) inexcitable cells, which displayed a low resting potential of about −35 mV (39% of cells tested, $n = 118$); and (2) excitable cells, which displayed either triggered or spontaneous action potentials and resting membrane potentials higher than −50 mV (61% of cells tested, $n = 185$).

5. In the light fraction from lactating females (second group), the majority of the cells were excitable (70%) and showed high resting membrane potentials (−50 to −55 mV) and 15% of these cells displayed spontaneous action potentials.

6. Heavy fractions (third group) contained a high percentage of non-spontaneous but excitable cells (80% of the cells tested, $n = 65$). These cells were able to elicit action potentials after the cessation of hyperpolarizing current pulses ('off' potentials).

7. Action potentials were insensitive to the sodium channel blocker, tetrodotoxin (TTX; $5 \times 10^{-6}$ M) but were reversibly blocked by calcium channel blockers such as cobalt, manganese and cadmium (10 mM).

8. In excitable cells from the three groups, dopamine ($10^{-7}$ M) induced a hyperpolarizing response due to an increase of the membrane conductance. During this response, action potentials were inhibited. It was shown that this was not a direct effect of dopamine. The reversal potential of the dopamine-induced response in these cells was found to be at −100 mV. This value was shifted to more positive potentials (−50 mV) when high-potassium medium was used (56 mM).

9. In non-excitable cells (first group), dopamine ($10^{-7}$ M) induced a hyper-

polarizing response due to a decrease of the membrane conductance. The amplitude of this response was dependent upon the membrane potential and the reversal potential was found to be near 0 mV.

10. Some of the non-spontaneous cells from the third group showed no membrane responses to dopamine but the 'off' spike was suppressed.

11. Because of the apparent implication of potassium currents in the dopamine response found in excitable cells, we attempted to characterize this response by using potassium current blockers. Cobalt and tetraethylammonium had no effect, whereas 4-aminopyridine induced a weak modification of the dopamine-induced response.

12. The dopamine response due to the increase of the membrane conductance was mimicked by a specific agonist of the D2 receptors (RU24926) and was blocked by specific dopaminergic antagonists (haloperidol and domperidone).

13. The physiological significance of the inhibition of electrical activity by dopamine is interpreted in terms of the stimulus–secretion coupling hypothesis.

## INTRODUCTION

Liberation of anterior pituitary hormones is known to be under the control of hypothalamic factors in such a way that, for each hormone produced by a specific cell type, there is a specific hypothalamic factor which stimulates release. Prolactin appears to be an exception to this general scheme since lactotrophs are the only anterior pituitary cell type which has no specific releasing factor and are, in contrast, under tonic inhibitory control (Meites & Clemens, 1972). Dopamine, the best known prolactin inhibiting factor is released into the median eminence from terminals of tubero-infundibular neurones, and the concentration of dopamine detected at this level is compatible with that required for effective *in vitro* inhibition of prolactin liberation (for review, see Ben-Jonathan, 1985). The mechanisms by which dopamine acts are largely unknown. However, there is evidence that it binds with a type D2 membrane receptor (Calabro & MacLeod, 1978; Kebabian & Calne, 1979; Enjalbert & Bockaert, 1983; Foord, Peters, Dieguez, Scanlon & Hall, 1983). It is believed that once dopamine has bound to its receptor, a complex is formed which is capable of transferring a signal to a (or the) second messenger(s) implicated in the secretory response: for example decrease in cyclic-AMP levels (Swennen & Denef, 1982; Schettini, Cronin & MacLeod, 1983) and/or modification of the phosphatidylinositol metabolism (Canonico, Valdenegro & MacLeod, 1983).

The demonstration of excitability (i.e. action potentials with a calcium component) in normal and tumoral prolactin-secreting cells (for review, see Douglas & Taraskevich, 1985), has given a new insight into the mechanisms by which liberation is coupled to stimuli, and has supported therefore, the hypothesis that dopamine may influence prolactin release by acting on the electrophysiological properties of these cells. Single-cell recordings have shown that dopamine can abolish spontaneous firing activity in teleostean prolactin cells (Taraskevich & Douglas, 1978). Because these studies were made with extracellular recordings, little is known about the direct membrane actions of dopamine. In tumoral cells (GH3), it has been shown that dopamine inhibited calcium action potentials (Dufy, Vincent, Fleury, Du Pasquier, Gourdji & Tixier-Vidal, 1979); however this effect was not well defined and fur-

thermore, it has been reported that these cells do not have high-affinity dopamine receptors (Cronin, Faure, Martial & Weiner, 1980).

Recent intracellular data obtained from human tumoral cells (Israel, Jaquet & Vincent, 1985) demonstrated that dopamine inhibited electrical activity by way of a hyperpolarizing response most probably mediated by an increase of the potassium conductance.

To date, there are few reports of studies dealing with lactotrophs obtained from lactating females. This is surprising since anterior pituitaries obtained from this source contain up to four times more lactotrophs than non-lactating donors (Kirk, 1986), and it is highly probable that these cells are in a functionally active state. With this in view, we chose these animals as cell donors and for comparison, identical trials were performed on cells of immature origin (14-day-old females).

In the present work, we report (1) the patterns of prolactin release and their response to dopamine in normal prolactin cells from different origins (14-day-old and lactating female rats) enriched by unit gravity sedimentation and maintained in primary culture; (2) the electrical properties displayed by these cells; and (3) the effects of dopamine on each of the electrical responses characterized.

A preliminary account of the data has been presented in abstract form before (Israel & Vincent, 1984).

## METHODS

### *Animals*

Anterior pituitary cells were obtained from Wistar rats housed in cages on a 15 h light–9 h dark cycle with food and water *ad libitum*. The animals used were either 14-day-old females or lactating females in the first week of lactation.

### *Preparation of the cells*

Cell dissociation was performed using the Hopkins & Farquhar (1973) technique as modified by Denef and colleagues (Denef, Hautekeete, De Wolf & Van Der Schueren, 1978) and involved an enzymatic treatment (0·5% trypsin) followed by mechanical dissociation with a flame-polished Pasteur pipette. Yields of $1{\cdot}1 \times 10^6$ cells per pituitary gland of 14-day-old females were obtained while glands from lactating females normally yielded 3·5 to $4{\cdot}5 \times 10^6$ viable cells per pituitary.

The cell-separation method has been described previously (Denef *et al.* 1978). Briefly, it consisted of layering cells onto 1·1 l of a continuous density gradient of 0·3–2·4% bovine serum albumin (BSA) and allowing the cells to sediment for 3 h. The initial 300 ml of the gradient which contained red blood cells and cellular debris were discarded and fractions of 100 ml were then collected and numbered 2–9. The fractions were centrifuged at 800 *g* for 10 min and each pellet was resuspended in Dulbecco's modified Eagle medium (DMEM) containing 0·3% BSA to give a cell density of about $5 \times 10^6$ cells per ml. Cells were plated onto untreated 35 mm Petri dishes (Nunc, Denmark) at $10^5$ cells per dish for electrophysiological experiments and $10^6$ cells per dish for liberation studies. The dishes were stored for 30 min in a humidified incubator (37 °C, air: 94%, $CO_2$: 6%) then medium (DMEM + 3·7 g $NaHCO_3$/l containing 10% new-born calf serum, 35 mg penicillin/l and 50 mg streptomycin/l, pH 7·4) was gently added and subsequently changed after the first 48 h in culture. Antibiotics were thereafter omitted and medium was renewed every 3 days. Liberation experiments were performed after 6 days *in vitro* and electrophysiological experiments after 10 days *in vitro*.

The degree of lactotroph enrichment was assessed by immunofluorescent staining of cultures.

### *Hormone-release studies*

Hormone-release experiments were carried out in the original Petri dishes after a light microscopic examination. A continuous-flow perifusion system which accommodated four cultures at a time was devised using a four-channel peristaltic pump (Minipuls, Gilson) and constant tem-

perature (37 ± 0·5 °C) was maintained by a water-heated platform. An air-tight Sylgard seal of the Petri dish covers ensured a constant flow of medium over the cells. The perifusion medium was DMEM + 15 mM-HEPES + 12 mM-$NaHCO_3$ except in experiments with high extracellular potassium in which the medium was as shown in Table 1. Medium levels remained at about 2 ml/dish.

TABLE 1. Composition of modified media

| | Working concentrations (mM) |
|---|---|
| Glucose | 5 |
| $NaH_2PO_4$ | 0·9 |
| Sodium pyruvate | 1 |
| HEPES (pH 7·2) | 15 |
| $NaHCO_3$ | 10 |
| KCl (1) | 5·5 |
| KCl (2) | 55 |
| NaCl (1) | 140 |
| NaCl (2) | 90 |
| $MgSO_4$ | 0·4 |
| $CaCl_2$ | 1·8 |

flow rate was 0·5 ml/min, dead time including fraction collection was in the order of 1 min and dead volume was about 1·5 ml. A two and a half hour stabilization period was allowed before beginning an experiment. Aliquots were collected during a 2 min sampling time in tubes containing 50 $\mu$l of phosphate buffer solution (PBS) + 1 % BSA, and stored at −20 °C until radioimmunoassay (r.i.a.).

*Assay and staining*

Prolactin liberation was measured by specific rat-prolactin r.i.a. with kits provided by the National Hormone and Pituitary Program (Baltimore, MD, U.S.A.). All samples were assayed in duplicate. Prolactin values were expressed as nanogram equivalents of rat standard prolactin-RP1 (NIAMDD).

Immunofluorescent staining of cultures was performed in the Petri dishes, according to the technique of Dubois (1972). Briefly, cells were fixed for 10–15 min in freshly prepared 4 % paraformaldehyde in PBS and washed three times in PBS. A 10 min incubation of the cells with 1/20 normal sheep serum in PBS was sufficient to saturate the non-specific antigenic sites. Following another three PBS washes, the cells were exposed to specific antisera (anti-rat prolactin, anti-rat adrenocorticotrophin, anti-rat growth hormone (GH), anti-rat luteinizing hormone (LH) and anti-rat thyrotrophin stimulating hormone (TSH) at a 1/100 or 1/200 dilution in PBS containing 0·5 % human serum albumin at 4 °C for 24 h. At the end of incubation, the cultures were again washed in PBS and reacted for 1 h at 4 °C with fluorescein–isothiocyanate-labelled anti-rabbit immunoglobulin G prepared as described by Dubois (1972). The cells were then prepared for examination using glycerine–PBS as a mounting medium and observed and photographed using a microscope with fluorescent light.

*Chemicals*

Culture medium (DMEM powder without $NaHCO_3$) and sera were from Gibco (95051 Cergy-Pontoise, France). RU24926 was a gift from Roussel Uclaf Laboratories (93230 Romainville, France). Spiperone and haloperidol were a gift from Janssen Chemica (Beerse, Belgium). Cobalt ($CoCl_2$), HEPES, manganese ($MnCl_2$), barium ($BaCl_2$), tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), dopamine and tetrodotoxin (TTX) were purchased from Sigma (Chemical Company, St. Louis, MO, U.S.A.).

*Intracellular recordings*

About 30 min before the recording session, the culture medium was replaced by a recording medium (DMEM + 12 mM-$NaHCO_3$ + 15 mM-HEPES). The temperature of the bathing solution was maintained at 36 ± 1 °C using a warm-air system. The cells were viewed through a phase-

contrast, inverted microscope (Nikon TMD). Intracellular micro-electrodes were made with a horizontal puller (Mecanex, Geneva) and had a tip diameter of less than 0·2 μm. Their resistance varied from 100 to 150 MΩ when filled with a solution of 4 M-potassium acetate. A single micro-electrode bridge amplifier (DAGAN Cell Explorer 8700) was used to record the potential of the cell tested and to inject transmembrane current.

Drugs were dissolved in the recording medium and were delivered through 1–2 μm micropipettes connected to an air pressure system and positioned close to the cell to be tested. We have also used large-diameter micropipettes (5–10 μm) which permits gravity flow of drugs. In this case, after each application, the micropipette was withdrawn from the medium to avoid leakage of drugs.

## RESULTS

### *Distribution of cells*

The percentage of lactotrophs found after immunofluorescent staining of cultures from any given fraction varied according to the type of animal used.

The separation profile of cells originating from 14-day-old females was as follows: fractions 2 and 4, 40–60% lactotrophs; fraction 3, 70–85%. These three fractions were generally pooled and immunofluorescent staining showed that corticotrophs (10–20%) and somatotrophs (5–10%) were the principal contaminants of the pool containing fractions 2–4. Fractions 5 and 6 contained less than 10% lactotrophs and fractions 7–9 almost no lactotrophs. These last fractions contained essentially basophils as has been previously reported by Denef *et al.* (1978).

Pituitary cells prepared from lactating females gave high lactotroph concentrations in fractions 2–5 with maximum numbers being found in fractions 3 and 4 (up to 95%). Other cells identified by immunofluorescent staining in fractions F2–F5 were shown to be principally corticotrophs. A second population of lactotrophs was found in the heavy fractions (F7–F9) of cells from lactating female donors which constituted 40–60% of the total cell count in those fractions. Somatotrophs and gonadotrophs represented 10–20% and 15–20%, respectively, of the cells in the fractions 7–9.

The cells to be examined were therefore repartitioned into three groups as follows: group 1 included gradient fractions 2–4 from 14-day-old female rats; groups 2 and 3 consisted of respectively gradient fractions 2–5 and fractions 7–9 from the lactating females.

### *Hormone release*

Perifusion experiments showed that detectable quantities of prolactin were continually secreted for periods of up to 4 h in all cells tested. Basal release from the cells of the first and the third groups was stable with mean release values of 10 ng prolactin $ml^{-1}$ 2 $min^{-1}$ $10^6$ $cells^{-1}$ (10·3 ± 6·5 ng, mean ± S.E. of mean, $n = 212$; 10·2 ± 5·4 ng, $n = 71$) while cells from the second group showed large fluctuations from one sample to another and mean release was 38·7 ± 19 ng prolactin $ml^{-1}$ 2 $min^{-1}$ $10^6$ $cells^{-1}$ ($n = 133$). Hormone release in all cells was stimulated by thyrotropin-releasing hormone in a dose-dependent manner from $10^{-10}$ to $10^{-6}$ M and by excess potassium (55 mM) (results not shown).

Responses obtained from the perifusion of dopamine-containing medium are shown in Fig. 1*A–D*. Prolactin release was inhibited in cells from the first group (Fig. 1*A*); however the dose–response curve obtained in these cells (Fig. 1*B*) showed

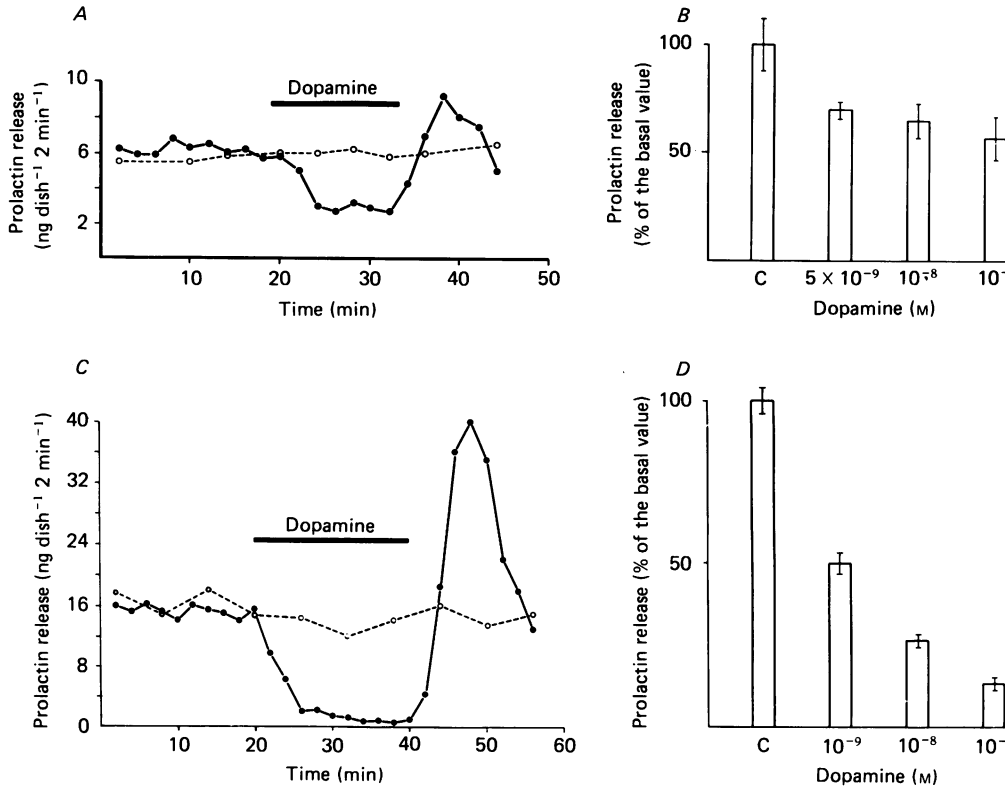


Fig. 1. Effect of dopamine on spontaneous prolactin release from normal lactotrophs in primary culture. *A*, cells from the first group (light fraction, 14-day-old females) were continuously perifused and medium was collected every 2 min (0·5 ml/min; 1 ml/aliquot) for dopamine treatment (●) or every 4 min for control (○). Dopamine ($10^{-7}$ M) was added for a 16 min period (bar). During dopamine treatment the basal level of prolactin was decreased to approximately 50% of the control. After the cessation of treatment, the prolactin release showed a slight rebound before returning to its basal level. *B*, dose–effect relation for dopamine in cells from the first group (C = control, mean ± S.E. of mean). This curve is representative of five experiments. *C*, effect of dopamine on the basal release of prolactin from cells of the second group (light fraction, lactating female). The protocol is the same as in *A*. Dopamine strongly inhibited prolactin release. Note the significant rebound of prolactin release following dopamine treatment. *D*, relationship between dopamine concentration and its inhibitory effect on prolactin release from the second group cells (C = control, mean ± S.E. of mean). This curve is representative of four experiments.

a rather weak effect (35% inhibition). On the other hand, the response obtained in cells from the second group was marked (up to 90% inhibition) both in a time *versus* release plot (Fig. 1*C*) and dose–effect curve (Fig. 1*D*). The post-inhibition rebound observed in cells of this origin (Fig. 1*C*) was frequent and is corroborated in reports by Denef and collaborators (Denef, Baes & Schramme, 1984). The third group of cells gave similarly shaped responses (not shown) but dopamine was less effective in inhibiting release (70% inhibition).

*Intracellular recordings*

*Resting properties.* After 8 days *in vitro*, one can expect to obtain stable recordings from cultured cells (see Israel, Denef & Vincent, 1983). After impalement, injured cells displayed a low membrane potential (−10 to −20 mV) which progressively

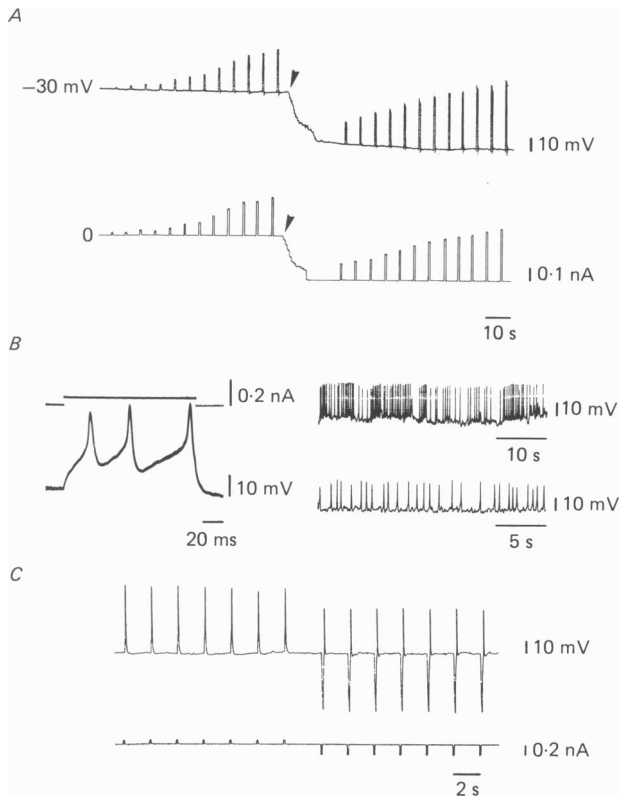


Fig. 2. Electrical activity recorded from presumptive lactotrophs. *A*, typical recording from an inexcitable cell from the first group (light fraction, 14-day-old females). The upper trace shows the membrane potential, the lower trace corresponds to injected current used to vary the membrane potential. When the cell was at resting potential (left), depolarizing current pulses of increasing amplitude did not trigger action potentials, nor when it was held at −65 mV (arrow) by a constant hyperpolarizing current (right). *B*, left: action potentials evoked by a depolarizing pulse (upper trace) in an excitable cell of the first group. Right: spontaneous activity recorded in a cell from the first group (upper right) and in a cell from the second group (light fraction, lactating females) (lower right). *C*, electrical activity recorded in a cell from the third group (heavy fraction, lactating females). Action potentials were elicited either by depolarizing pulses (left) or followed hyperpolarizing pulses ('off' potentials; right).

decreased to 0 mV. Only those cells which displayed a stable potential for more than 5 min were used in this study.

In total, 495 cells were recorded with 303 cells from the first group, 127 cells from the second group and 65 cells from the third group.

Regardless of their donor origin, cells could be separated according to their ability

or inability to produce action potentials. Most cells were excitable: first group, 61% of all cells tested ($n = 303$); second and third groups, about 75% of all cells tested ($n = 192$). The remaining cells from the first group were non-excitable and displayed low resting potentials ($-32{\cdot}2 \pm 5{\cdot}4$ mV; mean ± S.E. of mean; $n = 17$) (Fig. 2*A*) with

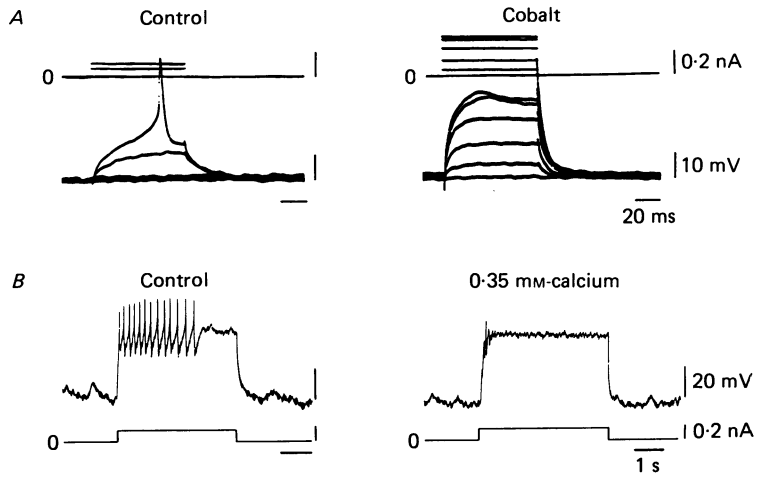


Fig. 3. Ionic properties of the action potentials: effect of cobalt and low-calcium medium. *A*, effect of cobalt. A control action potential (left) was triggered by a depolarizing current pulse (upper trace). This action potential was totally blocked (right) during the application of cobalt ions (10 mM in the delivery pipette). *B*, effect of low-calcium medium. Left: a burst of action potentials was triggered by a long depolarizing pulse (5 s) in a control medium (1·8 mM-calcium). Right: an identical stimulation was delivered in a modified recording medium with reduced calcium concentrations (control calcium = 1·8 mM; reduced calcium = 0·35 mM). In this condition, no burst could be triggered except for a brief response at the beginning of the depolarizing pulse.

an input resistance of $175 \pm 51$ MΩ (mean ± S.E. of mean; $n = 12$). That these cells were not excitable can be explained by their relatively low membrane potential. Moreover, when this type of cell was held hyperpolarized to $-60$ mV by constant transmembrane current, depolarizing current pulses still did not elicit action potentials (Fig. 2*A*).

The resting potential ($-55 \pm 7{\cdot}9$ mV, $n = 15$) and membrane resistance ($224 \pm 33$ MΩ, $n = 11$) were greater in excitable cells than in non-excitable cells. Only a few of the cells tested (15%) displayed spontaneous action potentials after micro-electrode penetration (Fig. 2*B*). Firing frequency increased as a function of depolarization, but never exceeded 2·5 Hz.

Single action potentials could be triggered in non-spontaneous cells by short (10 ms) depolarizing current pulses while long-lasting pulses (150 ms) elicited bursts (Fig. 2*B*). In some cells from the third group, it was possible to elicit action potentials after the cessation of hyperpolarizing pulse (Fig. 2*C*). These action potentials were named 'off' potentials.

The amplitude of the action potentials ranged from $30{\cdot}8 \pm 3{\cdot}1$ mV (spontaneous action potentials, $n = 160$) to $39{\cdot}4 \pm 3{\cdot}4$ mV (triggered action potentials, $n = 140$) (Fig. 3). No differences were detected in their duration ($12{\cdot}8 \pm 2{\cdot}1$ ms, $n = 42$).

*Action potentials.* To determine the ionic mechanisms responsible for the action potentials, we have used classical blockers. The calcium current inhibitor, cobalt, inhibited the action potentials (Fig. 3*A*) in a reversible manner. We have also tested the effect of decreasing concentrations of external calcium and it was seen that a 5-

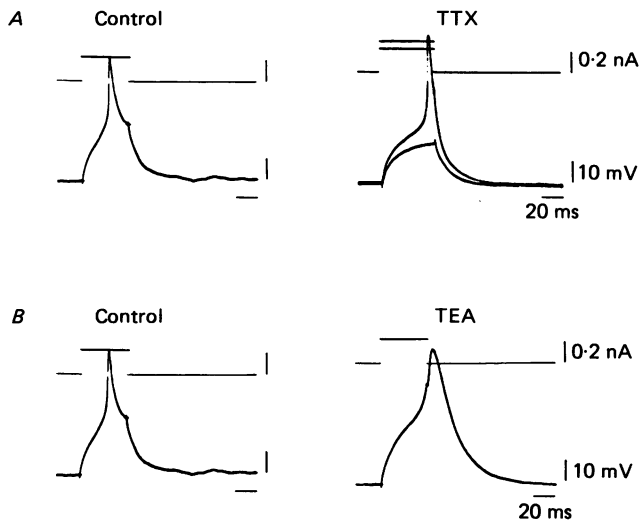


Fig. 4. Ionic properties of the action potentials: effect of tetrodotoxin and tetraethylammonium. *A*, effect of tetrodotoxin (TTX). Left: a control action potential was recorded in a normal medium. Right: TTX ($5 \times 10^{-6}$ M) ejected in the vicinity of the cell did not alter the action potential. *B*, effect of tetraethylammonium (TEA). Left: the control action potential is the same as in *A*. Right: an ejection of TEA (10 mM) prolonged significantly the duration of the action potential.

fold decrease in calcium ions (1·8–0·35 mM; calcium ions were replaced by magnesium ions) suppressed the action potentials (Fig. 3*B*). Selective inhibition of sodium currents by a high concentration of TTX ($5 \times 10^{-6}$ M) had no effect either on the amplitude or on the duration of action potentials (Fig. 4*A*). However, at such a concentration, TTX slightly modified the input membrane resistance. This is why the amplitude of the depolarizing pulse has to be slightly increased to be effective (Fig. 4*A*).

Tetraethylammonium (TEA), which is known to inhibit the potassium current responsible for repolarization, markedly increased the duration of the action potentials from $12 \pm 1{\cdot}4$ to $40 \pm 6{\cdot}4$ ms ($n = 8$) (Fig. 4*B*).

*Effects of dopamine on the electrical properties of the cells*

In all the experiments reported here, dopamine ($10^{-8}$ or $10^{-7}$ M) was applied directly to the cell via a glass delivery pipette. Because these cells were very fragile and sensitive to pressure artifacts, we have generally applied substances by unit gravity (see Methods).

According to the electrical properties of the cell tested, one of three types of response was possible following the application of dopamine.

*Dopamine response induced in excitable cells.* In all excitable cells from the three

groups, with the exception of cells which displayed 'off' potentials, dopamine elicited a hyperpolarizing response which was concomitant with a decrease in the input membrane resistance (Fig. 5*A*) and action potentials no longer occurred (Fig. 5*B* and *C*).

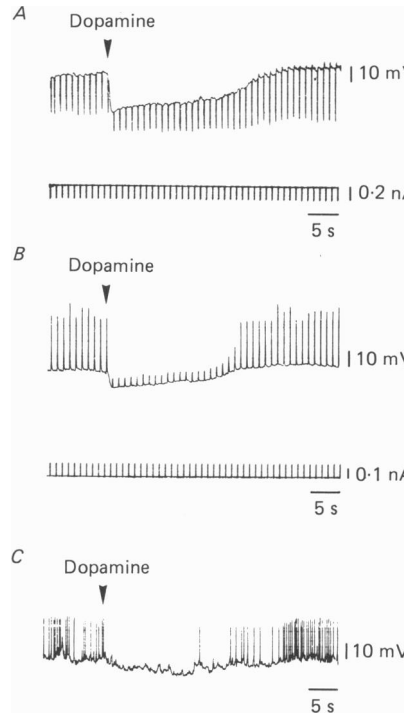


Fig. 5. Effect of dopamine on the electrical properties of excitable cells. *A*, the cell studied (first group, light fraction, 14-day-old females) was at resting potential (−55 mV). Dopamine ($10^{-7}$ M in the delivery pipette) induced a hyperpolarizing response characterized by a decrease of the membrane resistance as indicated by the attenuation of the deflections induced by hyperpolarizing currents (lower trace, 0·2 nA, 100 ms, 1 Hz). The dopamine-induced response lasted for several seconds. *B*, action potentials were triggered by depolarizing pulses (lower trace), and were suppressed during the dopamine response. Only the passive depolarizing responses remained. *C*, effect of dopamine on a spontaneously firing cell (resting potential: −50 mV). Action potentials were totally abolished under dopamine treatment.

We examined whether the absence of action potentials after dopamine was due to a direct or indirect effect by testing the excitability of cells during dopamine treatment. In spontaneously active cells, action potentials were suppressed by dopamine but discharge reappeared when a constant depolarizing current was applied (Fig. 6*A*). The same effect occurred in non-spontaneously active cells, after hyperpolarization by dopamine, when the amplitude of the depolarizing current pulses was increased (Fig. 6*B*). These two observations suggest that dopamine does not have a direct inhibitory effect on the formation of action potentials.

To determine if the amplitude of the hyperpolarizing response induced by dopamine was dose dependent, a thin micropipette (1–2 $\mu$m diameter) was used and ejection time was controlled by a stimulator. As illustrated in Fig. 7, the amplitude

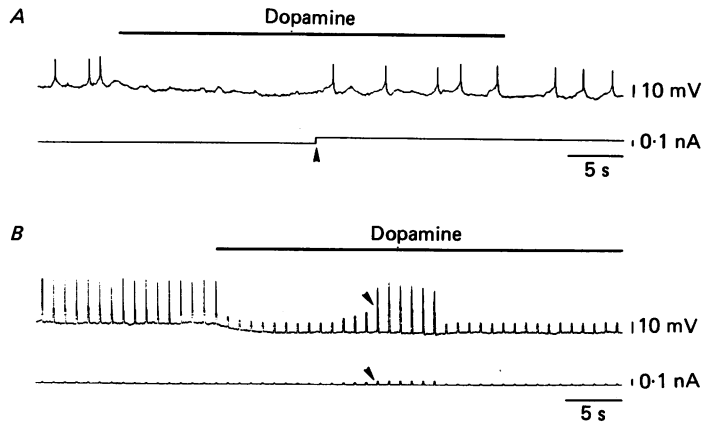


Fig. 6. Lack of a direct inhibiting effect of dopamine on action potentials. *A*, constant application of dopamine ($10^{-8}$ M in the local delivery pipette) induced a slight hyperpolarizing response which led to the inhibition of spontaneous action potentials. During dopamine treatment, a small depolarizing current (lower trace, arrowhead) induced a slight depolarization which was sufficient to restore firing. *B*, action potentials were triggered by small depolarizing current pulses (0·05 nA, 100 ms, 1 Hz). Dopamine ($10^{-8}$ M) induced a hyperpolarizing response which inhibited action potentials (only the passive membrane response remained). When the amplitude of the depolarizing pulses was sufficiently increased (lower trace, arrowhead), the cell began firing again (arrowhead).

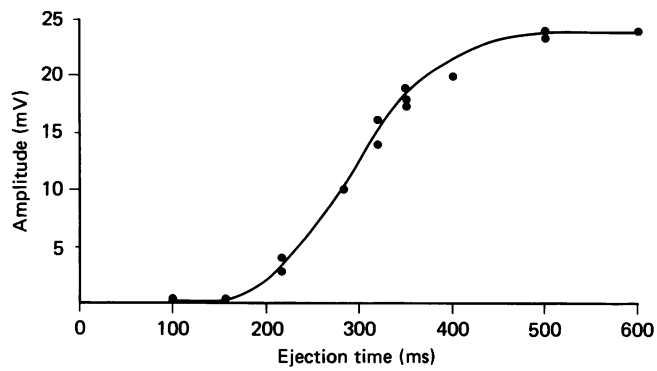


Fig. 7. Dose–response relationship for dopamine. Dopamine was delivered from a thin micropipette (1–2 $\mu$m) close to a single cell (ejection time varied from 100 to 600 ms). The absolute amplitude of the induced responses (hyperpolarizations) was plotted *versus* the duration of ejection. The resting potential of the cell was −55 mV.

of the dopamine response was correlated with dopamine ejection time; however, a plateau is observed with longer application times (500 ms).

The amplitude of the response to dopamine in these cells increased with depolarization, decreased with hyperpolarization and the reversal potential was found to be near −100 mV (−103±4 mV; $n = 6$) (Fig. 8*A*). The current–voltage relationship was analysed in normal conditions and during dopamine application. The two current–voltage plots crossed the abscissa at about the same value (mean = −100±3 mV; $n = 4$) (Fig. 8*B*).

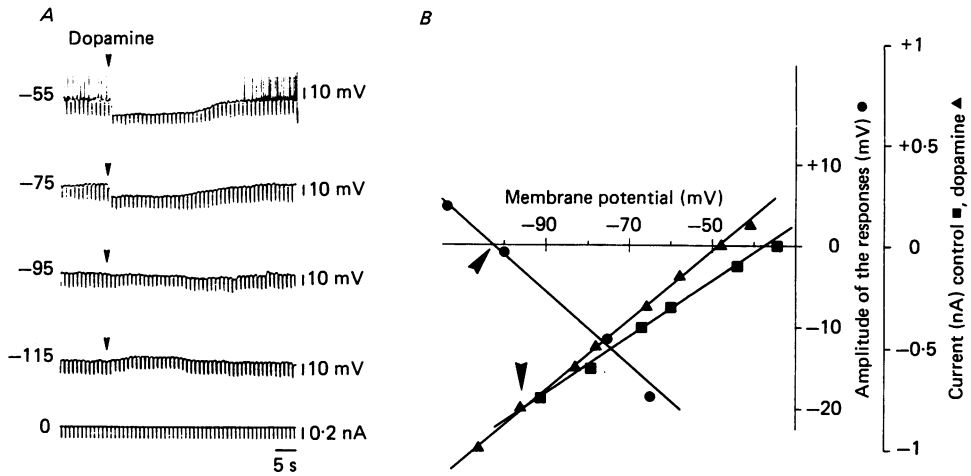


Fig. 8. Ionic properties of the dopamine-induced response in excitable cells. *A*, dopamine was tested at different membrane potentials (−55, −75, −95 and −115 mV) on the same cell (excitable cell, light fraction, 14-day-old females). The amplitude of the responses decreased as the cell was hyperpolarized and was reversed at high membrane potentials. *B*, the amplitude of the dopamine response (●) was plotted *versus* the membrane potential and the reversal potential was found to be near −100 mV (arrowhead). The current–voltage relations were performed in normal medium (■) and in dopamine-containing medium (▲). The two plots crossed at the same potential (−98 mV, arrowhead).

The resulting reversal potential suggested the participation of potassium ions. For this reason, the effects of two high-potassium solutions (30 and 56 mM) on the reversal potential of the dopamine-induced response were tested (in this case, dopamine was dissolved in the high-potassium-containing medium). With high-potassium medium, the reversal potential was shifted to more positive values (mean = $-70 \pm 3$ mV for 30 mM-potassium solution and $-55 \pm 3$ mV for the 56 mM-potassium solution) (Fig. 9).

In order to characterize more precisely the type of potassium current implicated in the dopamine response, we tested the effects of 4-AP and TEA in the presence of dopamine as well as the dependency of potassium current on calcium by adding cobalt to the test media. All substances were applied before or during dopamine application and the results were as follows.

(i) Four concentrations of TEA were employed (10, 30, 50 and 100 mM). At concentrations of 30 mM or more, TEA induced a slight depolarization of the resting membrane potential (up to 10–15 mV) but did not alter the dopamine response (Fig. 10*A*).

(ii) Two concentrations of 4-AP (1 and 5 mM) were used and, as shown in Fig. 10*B*, this substance slightly altered the dopamine-induced responses (depolarization of 6 mV).

(iii) Cobalt was used at a concentration of 10 mM and, as seen in Fig. 10*C*, it induced a modification of the resting membrane resistance but did not alter the dopamine response.

*Dopamine response induced in non-excitable cells.* Dopamine induced a hyper-

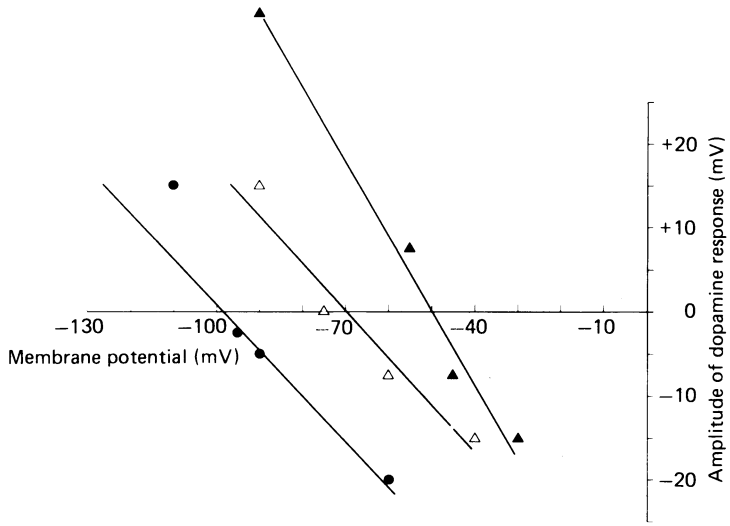


Fig. 9. Effects of high external potassium on the reversal potential of the dopamine response. This experiment was performed in normal medium (●) and in high-potassium medium (30 mM, △ and 55 mM, ▲). It was found that the dopamine response in 30 mM-potassium medium had a reversal potential close to −70 mV, while in 56 mM-potassium, the reversal potential was found to be near −50 mV.

polarizing response varying from 10 to 30 mV amplitude in non-excitable cells (Fig. 11*A*). This response lasted for 10–20 s and desensitization was not observed after successive applications. In contrast to the response observed in excitable cells, this response was due to an increase in the membrane resistance (Fig. 11*B*).

The amplitude of the dopamine response was dependent on the membrane potential of the cell, such that it increased when the cell was hyperpolarized and decreased when the cell was depolarized (Fig. 11*C*). The reversal potential of the response was found to be near 0 mV (Fig. 11*D*).

*Dopamine response induced in cells which show 'off' action potentials.* In these cells of the third group, dopamine had no effect on the resting potential or on the input membrane resistance (Fig. 12*A* and *B*) nor did it have an effect on the action potentials triggered by depolarizing current pulses (Fig. 12*B*). It did, however, strongly inhibit 'off' spikes (Fig. 12*A*).

*Pharmacological study of the dopamine-induced response*

Pharmacological studies were performed only on excitable cells which responded to dopamine in order to test the specificity of this response.

*Effects of dopaminergic receptor agonists.* We have tested the non-catechol, non-ergot, dopamine agonist, RU24926 ($10^{-7}$ M) which is specific to D2 receptors (Enjalbert & Bockaert, 1983) and found a response comparable to that produced by dopamine. In both cases, an increased membrane conductance produced a hyperpolarization (Fig. 13*A* and *B*) with a reversal potential of about −100 mV (Fig. 13*C*).

*Effects of dopaminergic receptor antagonists.* Haloperidol and spiperone, two potent

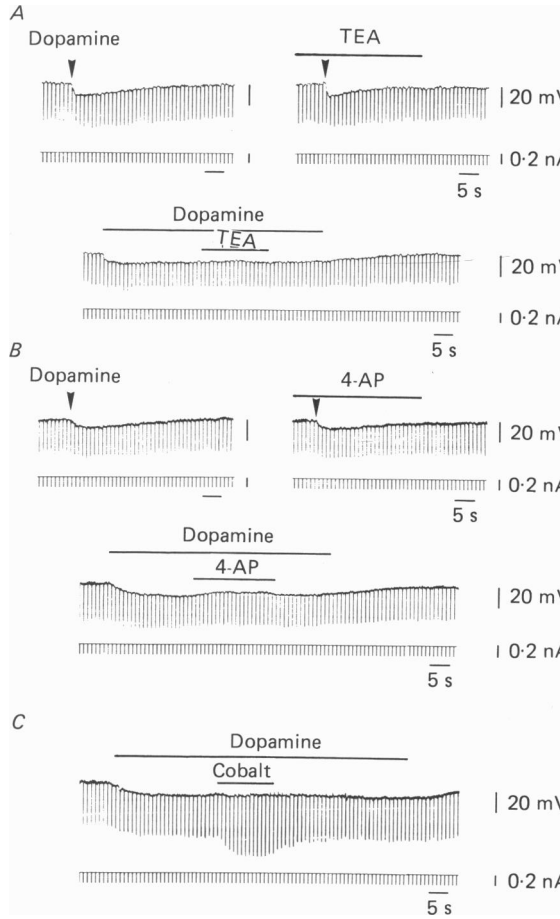


Fig. 10. Pharmacological study of the dopamine response induced on an excitable cell. These experiments were performed on excitable cells (light fraction, lactating females). *A*, effect of tetraethylammonium (TEA). Upper part, left: a typical response induced by a short application of dopamine (arrow) in control conditions. Right: an identical ejection of dopamine under a constant ejection of TEA (30 mM in the delivery pipette). Note the slight increase of the membrane resistance due to the TEA application. This record shows that TEA did not affect the dopamine response. Lower part: dopamine was applied for a period of 55 s. TEA (30 mM) was subsequently applied during dopamine treatment. TEA produced a minimal effect on the membrane potential. *B*, effect of 4-aminopyridine (4-AP). The protocol used was the same as described in *A*. Upper part, left: dopamine response induced in normal conditions. Right: dopamine response during 4-AP treatment (5 mM): no modification of the dopamine-induced response was observed. Lower part: application of 4-AP during a long dopamine treatment (50 s). Only a slight effect is seen. *C*, effect of a calcium current inhibitor. Cobalt (10 mM) was ejected during a long dopamine treatment. An increase of the membrane resistance was seen but no modification in the dopamine-induced response occurred.

dopamine antagonists (see Denef & Follebouckt, 1978) were tested in the following manner. Following a control application of dopamine, the specific drug was applied for a duration of 100 ms a few seconds before dopamine was reapplied. A comparison was made between the effect of dopamine with and without the antagonist. It was seen that neither antagonist had an effect on the passive electrical membrane

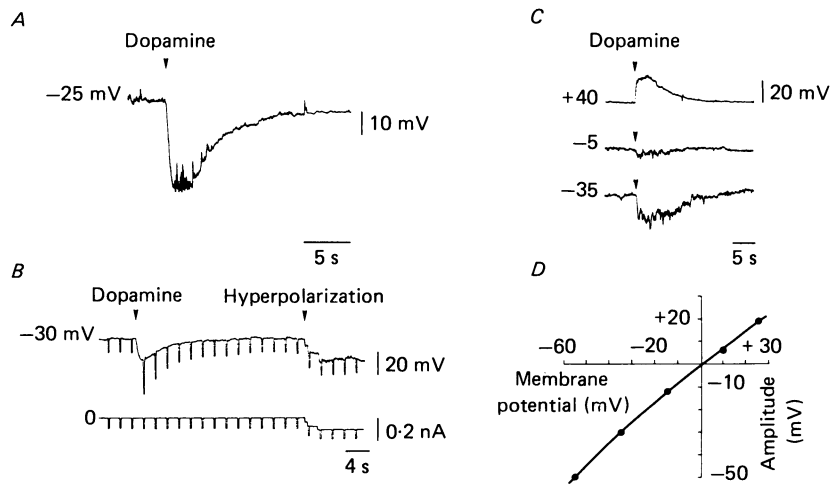


Fig. 11. Effect of dopamine on the electrical properties of a non-excitable cell. *A*, when the cell (light fraction, 14-day-old females) was at resting potential (−25 mV), dopamine (dopamine, $10^{-7}$ M) induced a significant hyperpolarizing response which lasted for several seconds. *B*, the effect of dopamine on the membrane resistance was tested by passing small hyperpolarizing current pulses (lower trace, 0·1 nA, 200 ms, 0·5 Hz). The voltage deflections induced by the current pulses were increased in the presence of dopamine indicating an increase of the membrane resistance. A hyperpolarization, following the dopamine treatment, induced by a constant current did not modify the membrane resistance. *C* and *D*, the dopamine-induced response was obtained at different membrane potentials (−35, −5 and +40 mV) (*C*) and its amplitude was plotted *versus* the membrane potential (*D*). The reversal potential of the response was found to be near 0 mV.

properties of the cell, but both substances completely blocked the dopamine response (Fig. 14).

## DISCUSSION

This study was undertaken to characterize in detail the response to dopamine of lactotroph cells in primary culture. However, our lactotroph-enriched culture contained a certain percentage of non-lactotroph cells, i.e. corticotrophs (less than 20%), somatotrophs (less than 10%) and thyrotrophs (less than 5%). It can be argued therefore that our recordings came from a heterogeneous population of cells, specially since somatotrophs and thyrotrophs are known to respond to dopamine. However, we can estimate that 65–80% of the cells in our culture were prolactin cells, and as we recorded a large sample of cells (up to 500), it is likely that the majority of the responses to dopamine were indeed recorded from lactotrophs. This contention is further supported by the fact that the responses recorded in this study were similar to the responses recorded from human pituitary tumour cells, which constituted a homogeneous population of prolactin cells (Israel *et al.* 1985).

An increase in cytosolic calcium has been shown to be essential to hormone release and several authors have suggested that calcium may enter these cells via specific channels and notably those channels implicated in the generation of electrical activity (action potential, membrane noise). In the light of such a hypothesis, the electrical membrane properties of several types of secretory cells, including pituitary

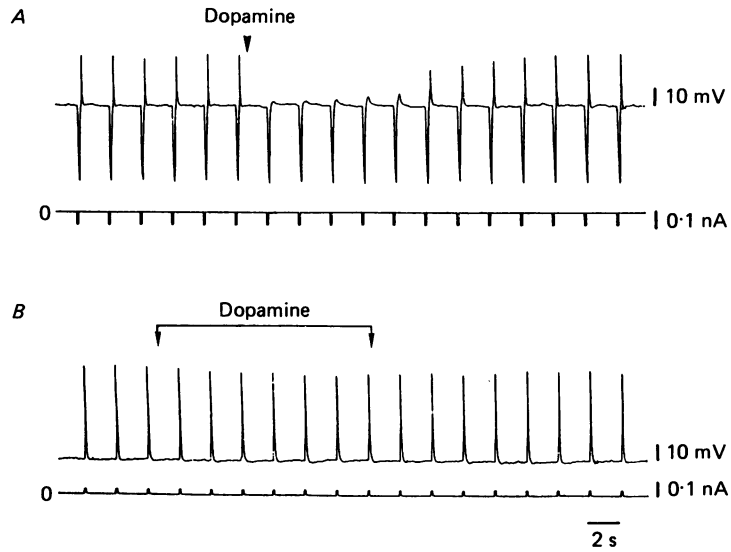


Fig. 12. Lack of effect of dopamine on resting potential and membrane resistance on cells which show 'off' action potentials. Action potentials were triggered either by passing hyperpolarizing (*A*, lower trace) or depolarizing current pulses (*B*, lower trace); (cell from heavy fraction, lactating female). Dopamine ($10^{-7}$ M in the delivery pipette) had no effect on the resting potential or on the membrane resistance; however, it inhibited 'off' potentials (*A*, upper trace) and was without effect on action potentials triggered by depolarizing current pulses (*B*, upper trace).

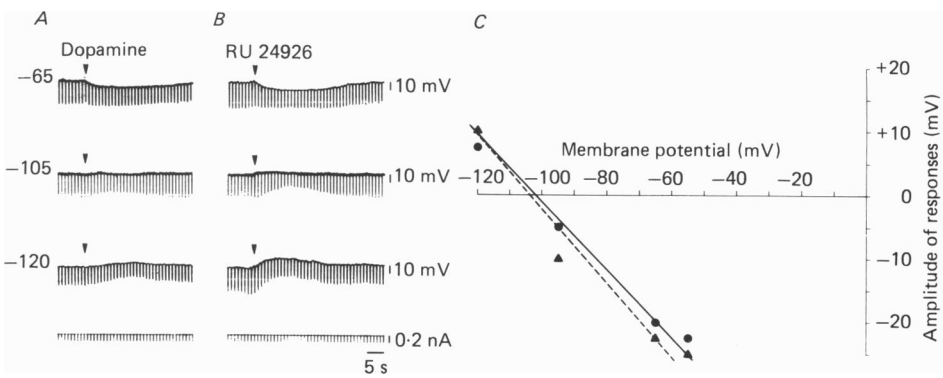


Fig. 13. Effect of the dopamine agonist RU24926 on the electrical properties of an excitable cell. The dopamine agonist RU24926 ($10^{-7}$ M in the delivery pipette) induced the same response (*B*) as dopamine (*A*) which consisted of a hyperpolarization associated with an increase of the membrane conductance (excitable cell from the light fraction, 14-day-old females). The amplitude of the responses for both RU24926 (▲) and dopamine (●) at different membrane potentials (−65, −105 and −120 mV) obtained from the same cell were plotted *versus* the membrane potential; the reversal potential of the responses was similar for both substances and was found to be near −105 mV (*C*).

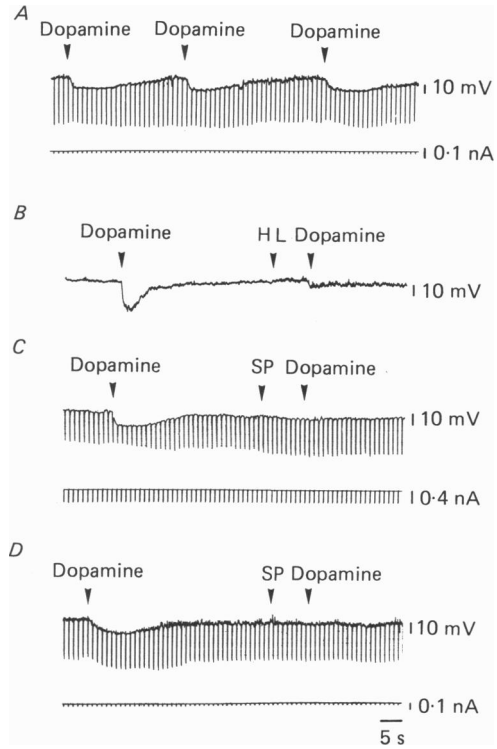


Fig. 14. Modification of the dopamine-induced response from an excitable cell by dopaminergic receptor antagonists. *A*, three successive applications of dopamine were delivered on the same cell (light fraction, 14-day-old females) and were able to induce responses of the same amplitude: no desensitization was observed. *B*, dopamine was tested on an excitable cell from the first group (light fraction, 14-day-old females) and shown to elicit a hyperpolarizing response which served as control. The dopamine receptor antagonist haloperidol (HL) was then applied ($10^{-6}$ M in the delivery pipette, ejection time: 100 ms) and a second dopamine administration followed some seconds later. As is seen, haloperidol had no effect on the resting potential of the cell but strongly inhibited the subsequent dopamine response. *C*, dopamine applied on an excitable cell from the second group, induced a hyperpolarizing response which was concomitant with an increase of the membrane conductance. Spiperone (SP; $10^{-8}$ M, ejection time: 100 ms) an effective specific D2 receptor antagonist, had no effect on the resting potential or the membrane resistance but totally blocked the dopamine response. *D*, spiperone was tested as above on a cell from the third group. Dopamine induced a hyperpolarizing response which was totally blocked by spiperone treatment.

cells, have been described (for review, see Douglas & Taraskevich, 1985; Mason & Ingram, 1986).

In the present study, analysis of the ionic currents involved in the action potentials of normal excitable cells suggests the existence of a strong inward calcium current. This is in agreement with results obtained from clonal cell lines (Dubinsky & Oxford, 1984; Matteson & Armstrong, 1984) and from normal somatotrophs in culture (Israel *et al.* 1983). It has been proposed that the calcium which enters the cell during the calcium action potential could increase the amount of cytosolic calcium and in this way contribute to the hormonal release process. In view of the relatively

large basal release of prolactin, the low percentage of spontaneously active cells (15%) is puzzling, but a similar observation was initially reported from a study on chromaffin cells (Brandt, Hagiwara, Kidokoro & Miyazaki, 1976). However, many spontaneously active cells cease firing after penetration: it can be explained by membrane injury because of the extreme fragility of the prolactin cell membrane, given that extracellular field recordings prior to impalement demonstrated a higher number of spontaneously active cells. Another explanation may be that a leakage of ions from the micro-electrode results in a tonic hyperpolarization of the cell.

As for the cells of the first group which remained unexcitable even after hyperpolarization, they may constitute a particular pool of immature cells, and may not be the result of poor impalements, in respect to their ability to give a response to dopamine.

Substances known to affect hormone release are suspected to exert their action via a modulation of the electrical activity in endocrine cells (for review, see Vincent & Dufy, 1982). Dopamine has been shown to inhibit spontaneous firing in tumoral prolactin cells (Dufy *et al.* 1979), in teleost prolactin cells (Taraskevich & Douglas, 1978) and in normal pars intermedia cells (Douglas & Taraskevich, 1978) although no mechanism has been proposed to explain this effect. The concentration of dopamine that we have used in our experiments was generally ten times lower than the dopamine concentration used by other workers (Douglas & Taraskevich, 1978, 1982; Taraskevich & Douglas, 1978) and corresponds to the physiological concentration found in portal blood (Gibbs & Neill, 1978; see George, Watanabe, Di Paolo, Falardeau, Labrie & Seeman, 1985).

Our study has shown that the dopamine response differed according to the origin of the prolactin cell. The hyperpolarizing response due to a decrease of the membrane conductance was recorded only in the non-excitable cells from immature female rats. Considering the high percentage of this cell type (approximately 40% of the cells recorded) and the age of the cell donors, it may be possible that these cells constitute a population of incompletely differentiated cells. In contrast, heavy cells from lactating females whose sole response to dopamine was an inhibition of the 'off' action potentials, may possibly be aged cells. This is corroborated by the fact that this type of cell was rarely observed in cell populations taken during the first post-partum week but was frequently found at the time of pup severage. The absence of subsequent dopamine-induced hyperpolarizing responses may reflect a loss of high-affinity dopaminergic receptors or uncoupling between the receptor and the ionic channel. However, the effect of dopamine on the 'off' spike may be explained by the inhibition of the low-threshold inward current component (see Dubinsky & Oxford, 1984) which is activated at low membrane potential and which could be responsible for activation of the 'off' potential.

The most frequently observed response to dopamine was a hyperpolarization concomitant with an increase in membrane conductance. We have characterized this response using ionic and pharmacological tests. The value obtained for the reversal potential (−100 mV) is identical to that previously reported for human tumoral prolactin cells (Israel *et al.* 1985) and similar to hippocampal pyramidal cells (−90 mV, Benardo & Prince, 1982). The observed shift in the potential in high external potassium (56 mM) strongly suggests the participation of potassium ions.

The characterization of the potassium current implicated in the dopamine response with specific blockers was unsuccessful. The inhibitors we have used are specific for the three classical types of potassium currents (Thompson, 1977; for review see Adams, Smith & Thompson, 1980): (i) the transient potassium current, also called $I_A$, is blocked specifically by 4-AP; this inhibitor, even at high concentration (5 mM), has only a slight effect on the dopamine response (see Fig. 11 *B*); (ii) the delayed potassium current shows two components: (1) a voltage-dependent current, named $I_K$, which is blocked by TEA; in our experimental conditions, TEA has no effect at 30 mM; (2) a potassium current activated by internal calcium ions, also called $I_C$. External treatment of the cell by cobalt (10 mM) did not alter the dopamine response. However, it would be more pertinent to chelate internal calcium with an appropriate chelator (EGTA). We have attempted such an experiment, but we were unsuccessful because of the fragility of the cells. Further studies using the patch-clamp technique may be helpful.

It is now known that dopamine acts via specific membrane receptors in prolactin cells (Caron, Beaulieu, Raymond, Gagne, Drouin, Lefkowitz & Labrie, 1978) that have been identified as D2 receptors (Enjalbert & Bockaert, 1983). In our study, the effects of the dopaminergic antagonists haloperidol and spiperone confirmed these findings. Similar effects of D2 antagonists were reported on human prolactin cells (Israel *et al.* 1985). D1 antagonists were not tested in our primary culture, but we previously reported that flupentixol had no effect on the dopamine-induced response in human prolactin cells (Israel *et al.* 1985).

Moreover, the fact that RU24926 mimicked the dopamine response is in favour of the conclusion that the electrical response we have described is due to an interaction with the high-affinity D2 receptor (see George *et al.* 1985).

The modification of the membrane potential in response to dopamine that we have observed may have a cumulative effect on the population of activated voltage-dependent calcium channels (Reuter, 1983). First, the absence of action potentials means that additional voltage-dependent calcium channels are no longer activated. Secondly, the increase in membrane potential leads to the inactivation of those channels which are normally open at the resting membrane potential. The net effect is a significant decrease in the level of cytosolic calcium and, hence, an inhibition of the hormone release process. This may be one possible mechanism for the inhibition of prolactin release by dopamine.

Furthermore, the D2 receptor has been reported to be negatively coupled to membrane adenylate cyclase, which itself may modulate cytosolic calcium levels (Jakobs, Aktories & Schultz, 1984). At physiological concentrations, dopamine decreased the level of intracellular cyclic AMP in prolactin cells (Swennen & Denef, 1982) and/or inhibited phosphatidylinositol turn-over (Canonico *et al.* 1983). However, the function of intracellular cyclic AMP and inositol triphosphate has been only partially elucidated (for inositol phosphates, see Michell, 1986) and some workers have postulated that cyclic AMP may simply play a regulatory role (Miyazaki, Reisine & Kebabian, 1984). Thus, there may be two separate mechanisms which influence hormone release via the common factor of cytosolic calcium: (1) dopamine-induced changes in membrane electrical activity coupled with a direct membrane change in cyclic AMP–inositol triphosphate levels or (2) alterations in the membrane

electrical activity alone, leading to a decrease in cytosolic calcium which in turn, entrains modifications in cyclic AMP–inositol triphosphate levels.

In conclusion, electrophysiological studies performed on normal enriched populations of prolactin cells have shown that differences in the dopamine-induced membrane response exist. Furthermore, it has been shown that one type of response predominates, but there are at least two other forms of responses. Hormone-release experiments showed differences as well. The most characteristic electrical response and most striking release-inhibiting effect are both seen in the second group cells. These cells constitute the major fraction of cells obtained from actively lactating female rats. These results suggest that the existence of different electrical responses to dopamine may be taken as an indicator of different physiological states of the lactotroph.

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