

# *A Dopaminergic Receptor in Adrenal Medulla as a Possible Site of Action for the Droperidol-evoked Hypertensive Response*

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Recently, an inhibitory dopaminergic receptor has been described that modulates catecholamine release from adrenal medulla. It has also been reported that low doses of droperidol increase arterial pressure in some patients with pheochromocytoma. The authors investigated whether an effect of droperidol on such a receptor could be one of the mechanisms involved in the hypertensive response. Isolated cat adrenal glands were perfused with Krebs-bicarbonate solution, and the catecholamine release was measured in the effluent. Then, the glands were stimulated by activation of the nicotinic receptor (nicotine, 5  $\mu\text{M}$ ), and the effect of low and high doses of droperidol and/or apomorphine on the catecholamine secretory responses evoked by nicotine was investigated. Low concentrations of droperidol (0.05  $\mu\text{M}$ ) (a dopaminergic antagonist) markedly increased the secretory response induced by nicotine whereas higher concentrations (50  $\mu\text{M}$ ) decreased it. Apomorphine (1  $\mu\text{M}$ ) (a dopaminergic agonist) inhibits the catecholamine release produced by nicotine, and this inhibitory effect was completely reversed by the lowest concentration of droperidol but not by the highest. In fact, the high concentration of droperidol further inhibited the catecholamine release induced by nicotine. The results suggest that the hypertensive responses evoked by low doses of droperidol in some patients with pheochromocytoma could be due to the inactivation of a dopaminergic inhibitory system present in the adrenal medulla that, under physiologic conditions, limits the amount of catecholamines released by the gland. Such an inhibitory mechanism could operate in an exaggerated manner in patients with pheochromocytoma. (Key words: Anesthetic, intravenous; droperidol. Anesthetic techniques: neuroleptoanesthesia. Sympathetic nervous system: adrenal medulla; catecholamine release; dopaminergic receptors.)

SEVERAL REPORTS have suggested that a hypertensive response might follow the administration of droperidol to some patients with pheochromocytoma.<sup>1-3</sup> In most cases, the hypertensive responses were observed when low doses of droperidol were used.<sup>1-4</sup> Although the specific reasons for this effect are not known, the fact that droperidol might increase catecholamine release from tumor cells or sympathetic nerve endings<sup>2,5</sup> and, in addition, inhibit the uptake of these amines into nerve terminals<sup>2,6</sup> or chromaffin granules,<sup>7</sup> suggest that droperidol could be directly involved in the hypertensive response.

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Recent findings from our laboratory<sup>8</sup> have shown that the dopaminergic agonists (dopamine or apomorphine) inhibit catecholamine release evoked by activation of nicotinic receptors in the cat adrenal medulla, and this inhibitory effect is reversed by dopaminergic antagonists (haloperidol or sulpiride). In addition, the dopaminergic antagonists, by themselves, increase catecholamine release induced by nicotine. These observations support the view that the adrenal chromaffin cells possess dopaminergic receptors that modulate the physiologic catecholamine secretory process triggered by activation of the nicotinic receptor. Although it is not known if adrenal chromaffin cells in a pheochromocytoma are under nicotinic control, the fact that some PC12 cells have nicotine receptors favors this possibility.<sup>9</sup>

The objective of this study was to investigate if, as previously shown for haloperidol,<sup>8</sup> droperidol also acts on the dopaminergic receptor present in the cat adrenal gland and if this mechanism would provide an additional explanation for the increment of catecholamine release and the hypertensive responses observed when low doses of droperidol are used clinically. Our results suggest that the hypertensive responses observed when low doses of droperidol were used could, in addition to the mechanisms,<sup>2,5-7</sup> mentioned earlier, be a consequence of removing a negative feedback mechanism activated by endogenous dopamine in the adrenal chromaffin cells that normally restricts catecholamine release.

## Methods

Cats of both sexes weighing 2.5-4 kg were anesthetized with ether followed by chloralose (70 mg  $\cdot$  kg<sup>-1</sup> iv), and the abdomen was opened by a midline incision. Both adrenal glands were removed after insertion of a cannula into the adrenolumbar vein and perfused in a retrograde direction through this vein by means of a perfusion pump. The gland was placed in a glass funnel, and the surface of the gland was covered with minute incisions made with a hypodermic needle. The isolated glands were perfused with Krebs-bicarbonate solution at room temperature, as described previously by García *et al.*<sup>10</sup> The perfusion rate was adjusted to 1 ml/min.

## PERFUSION MEDIA

The normal Krebs-bicarbonate solution had the following composition (mM): NaCl, 119; KCl, 4.7; CaCl<sub>2</sub>,

2.5;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1.2;  $\text{NaHCO}_3$ , 25;  $\text{KH}_2\text{PO}_4$  1.2; and glucose, 11. This solution was equilibrated with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ , and final pH being 7.4. High  $\text{K}^+$  solution (140 mM) was made by adding KCl to Krebs-bicarbonate solution and reducing NaCl to maintain the tonicity between normal limits.

#### COLLECTION OF PERFUSATE SAMPLES

After 60 min of initial perfusion with Krebs-bicarbonate solution, collection of perfusate samples at 2-min intervals was initiated. The first two samples were collected to determine the spontaneous catecholamine output. Nicotine (a classical nicotinic receptor-stimulating agent) enhanced catecholamine release in a concentration-dependent manner. A concentration of nicotine that represents the approximate median effective concentration ( $\text{EC}_{50}$ ) for catecholamine release was selected and used throughout. Thus, a nicotine pulse (5  $\mu\text{M}$  for 2 min) was applied to the gland, and the total catecholamine output evoked by nicotinic stimulation during the 2-min pulse and 6-min thereafter was collected, measured, and named  $S_1$ . After a 40-min washout period with normal Krebs solution, a second and similar stimulus with nicotine was applied to the gland. The collection of samples was carried out identically to the first stimulus and named  $S_2$ . The reason for using nicotine instead of acetylcholine (the physiologic neurotransmitter) for stimulating catecholamine secretion in adrenal medulla is due to the fact that although the presence of a muscarinic receptor has already been demonstrated, its role on adrenal secretion is not well known at present, and the secretory effect of acetylcholine has been found to be related exclusively to nicotinic receptor stimulation.<sup>11,12</sup>

The experimental design always ended with a 2-min perfusion of the gland with high  $\text{K}^+$  (140 mM) in order to check the functional viability of the gland as far as its catecholamine secretory response was concerned.

To test their effects on catecholamine secretory response, droperidol (0.05, 0.5, 5, and 50  $\mu\text{M}$ ) or apomorphine (1  $\mu\text{M}$ ) were present, respectively, in the perfusion fluid 20 and 10 min before the second nicotine pulse, during the 2-min pulse of nicotine, and 6 min thereafter. When both droperidol and apomorphine were used, droperidol was present 20 min before the second nicotine pulse. Ten minutes later and in the presence of droperidol, apomorphine was incorporated into the perfusion medium. Both drugs were also present during the 2-min nicotine pulse and 6 min thereafter.

#### CATECHOLAMINE ASSAY

Total catecholamine content of perfusate samples (noradrenaline plus adrenaline) was determined according to Shellenberger and Gordon<sup>13</sup> without further purifi-

cation of alumina. Although initially designed for application to brain tissue, this method has also been used to determine catecholamine contents of plasma, urine,<sup>13</sup> and perfusion media.<sup>8,14-16</sup> Aliquots of perfusate were cooled and immediately acidified with perchloric acid to a final acid concentration of 0.05 N. The iodine reagent was used in the oxidation procedure. Under these conditions, the fluorescence of norepinephrine and epinephrine measured in a spectrophotofluorometer, Aminco-Bowman® (activation peak 380 nm, fluorescence at 495 nm) at room temperature was stable and reproducible. The sensitivity of the method for catecholamine metabolites is extremely low, and their presence did not interfere with the fluorescence developed by norepinephrine and/or epinephrine. On the other hand, in our experiments we measure only the release of endogenous catecholamines; because this process is the result of an exocytotic mechanism, one would not expect metabolites to be present in the effluent of adrenal gland which, in addition, lacks the postsynaptic type of cells that contribute to the metabolism of norepinephrine released at sympathetic junctions.

Catecholamines present in each collected sample were expressed as  $\mu\text{g} \cdot 2 \text{ min}^{-1}$  perfusion period. Net release of catecholamines evoked by nicotine during  $S_1$  or  $S_2$  was calculated by subtracting the spontaneous release from the release obtained during the 2-min nicotine pulse plus three more additional 2-min samples, and expressed as  $\mu\text{g} \cdot 8 \text{ min}^{-1}$ .  $S_2/S_1$  ratios were expressed as means  $\pm$  SE of the ratios obtained in each individual experiment with identical protocol.

#### DRUGS USED

The following drugs were used: apomorphine ClH (Sigma), dehydrobenzperidol (droperidol; kindly supplied by Dr. J. M. Moreno Alba from Sintex Latino, Madrid, Spain), and nicotine (Sigma).

#### STATISTICAL ANALYSIS

The data were expressed as means  $\pm$  SE. The statistical significance of the difference between means was determined by Student's *t* test for paired or group data.

#### Results

##### CATECHOLAMINE RELEASE EVOKED BY NICOTINIC RECEPTOR STIMULATION IN THE CAT ADRENAL GLAND

Spontaneous catecholamine release from cat adrenal glands was as low as  $173 \pm 20 \text{ ng} \cdot 8 \text{ min}^{-1}$  ( $n = 60$ ). When nicotine (5  $\mu\text{M}$  for 2 min) was perfused through the gland, it evoked a marked secretory response that peaked during the 2-min sample that followed the stimulus. Forty minutes

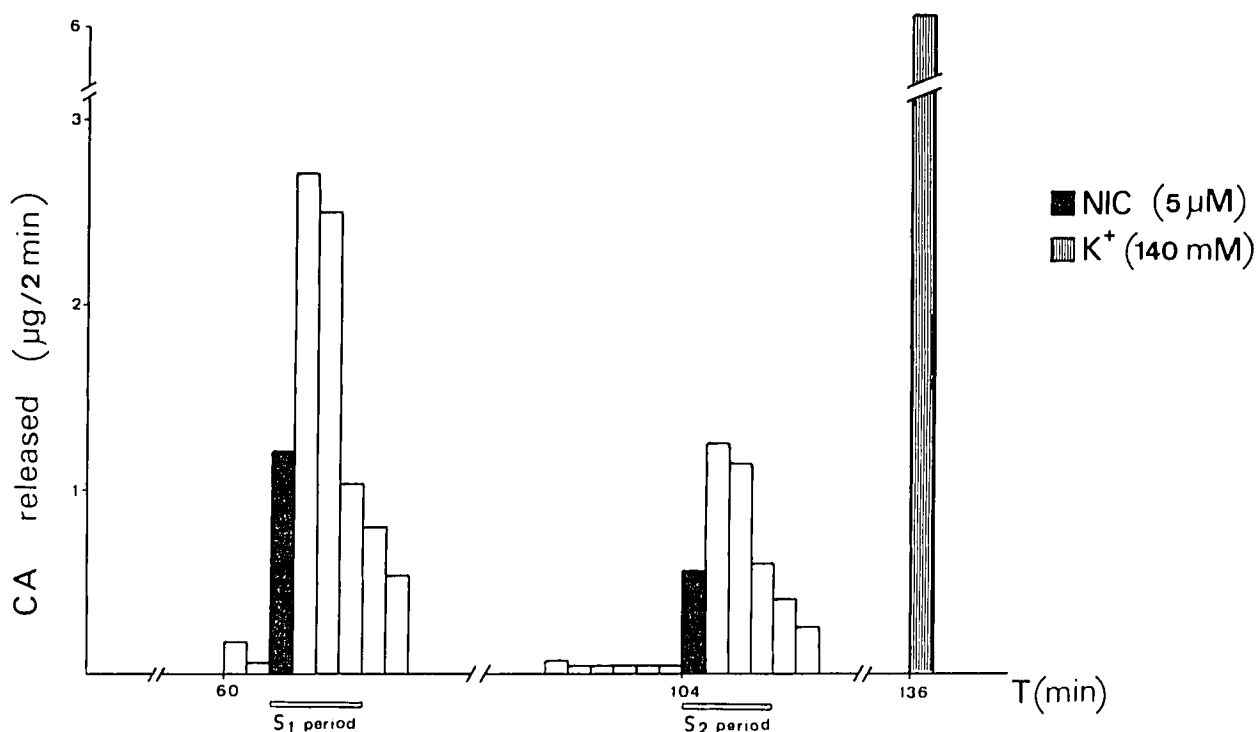


FIG. 1. Time-course of the catecholamine (CA) release evoked by two separate pulses of nicotine (NIC). The figure represents the release of CA obtained in each individually collected 2-min samples expressed in  $\mu\text{g} \cdot 2 \text{ min}^{-1}$ . Two nicotine pulses ( $5 \mu\text{M}$  for 2 min) were given 40 min apart and plotted as filled bars. The graph shows a typical experiment (out of 15). The striped bar indicates the release of CA induced by a 2-min pulse of high  $\text{K}^+$  (140 mM) solution.

later, a second pulse with nicotine was given (fig. 1). The net CA release obtained during  $S_1$  was  $7.64 \pm 1.14 \mu\text{g} \cdot 8 \text{ min}^{-1}$ . The release during  $S_2$  was reduced to  $3.55 \pm 0.46 \mu\text{g} \cdot 8 \text{ min}^{-1}$ , approximately 50% of  $S_1$  ( $n = 15$ ). ( $P < 0.001$ , paired comparison).

At the end of the experiment, a large catecholamine release was obtained when high  $\text{K}^+$  solution (140 mM for 2 min) was perfused (fig. 1), indicating that the gland was still functionally viable as far as the catecholamine secretory response was concerned.

#### EFFECT OF DROPERIDOL ON THE RELEASE OF CATECHOLAMINES EVOKED BY NICOTINE

Results are plotted in figure 2 as ratios of catecholamine secreted in  $S_2$  versus  $S_1$  and indicate that in the presence of the lowest concentration of droperidol ( $0.05 \mu\text{M}$ ), the  $S_2/S_1$  ratio was markedly increased ( $0.94 \pm 0.05$ ;  $P < 0.05$ ) as compared with the control nondroperidol-treated glands ( $0.50 \pm 0.03$ ). On the other hand, a droperidol concentration of 0.5 or  $5 \mu\text{M}$  did not modify the control values. However, when the higher concentration of the drug ( $50 \mu\text{M}$ ) was assayed, the catecholamine secretory response evoked by the second pulse of nicotine was almost completely abolished. None of the concentra-

tions of droperidol used alone modified the spontaneous release of catecholamine from the gland.

#### EFFECT OF DROPERIDOL ON THE INHIBITION BY APOMORPHINE OF THE CATECHOLAMINE SECRETORY RESPONSE INDUCED BY NICOTINE

On the basis of the different behavior of low and high concentrations of droperidol on catecholamine secretory responses evoked by nicotine, it seemed appropriate to investigate the effect of different concentrations of droperidol on the inhibitory effect of apomorphine on catecholamine release induced by nicotine. The results show that the inhibitory effect of apomorphine on catecholamine release evoked by nicotine was completely reversed when the low concentration ( $0.5 \mu\text{M}$ ) of droperidol was used (fig. 3). The  $S_2/S_1$  ratio in this case was  $0.62 \pm 0.05$ , a value significantly different ( $P < 0.01$ ) from control glands treated only with apomorphine, where the  $S_2/S_1$  ratio was  $0.16 \pm 0.03$ . In contrast, higher concentrations of droperidol ( $50 \mu\text{M}$ ) did not reverse the inhibitory effect of apomorphine on the secretory response evoked by nicotine; in fact, catecholamine release in this last case was almost completely abolished (fig. 3).

Discussion

The demonstration by Carlsson *et al.*<sup>17</sup> that in the central nervous system dopamine is not only the precursor of noradrenaline, but a transmitter on its own, greatly stimulated the research and characterization of dopamine receptors. More recently, specific peripheral dopamine receptors have been characterized in different neuronal and extraneuronal tissues such as vascular beds,<sup>18</sup> cell bodies of sympathetic neurones,<sup>19,20</sup> and noradrenergic nerve endings.<sup>21,22</sup> Recent results from our laboratory have also shown the presence of a specific peripheral dopaminergic receptor localized on the membrane of adrenal chromaffin cells, which modulates catecholamine release evoked by activation of the nicotinic receptor; when this receptor is activated by dopaminergic agonists, the catecholamine secretory response induced by nicotine in the cat adrenal gland is markedly reduced. Such inhibitory effect is completely reversed by dopaminergic antagonists, and it is not modified by the alpha-adrenergic blocking agent phentolamine, or by the opiate antagonist naloxone. In addition, haloperidol, by itself, increased catecholamine release evoked by nicotine.<sup>8</sup> Taken together, these data suggest the presence in the adrenal medulla of a dopaminergic tone normally maintained by endogenous dopamine that might be modulating the physiologic catecholamine release.

Because haloperidol blocks the dopaminergic receptor of adrenal medulla,<sup>8</sup> one would expect that droperidol, another drug of the butyrophenone group, could act in a similar manner and therefore contribute (among other possible mechanisms<sup>2,5-7</sup> to the hypertension occasionally seen during neuroleptoanaesthesia in patients suffering pheochromocytoma. The present results show that this might indeed be the case. Control experiments show that when two nicotine pulses were applied to the gland 40 min apart (fig. 1 and table 1), the net catecholamine release induced by nicotine during S<sub>2</sub> was only 50% of that found in S<sub>1</sub>. The reduction of the secretory response observed during S<sub>2</sub> is probably due to desensitization of the nicotinic receptor after its sustained exposure to the agonist<sup>23,24</sup> and not to tissue catecholamine depletion, because a pulse of high K<sup>+</sup> (140 mM) applied to the gland at the end of the experiment still induced a vigorous catecholamine secretory response. The presence of apomorphine reduced catecholamine release evoked by nicotine during S<sub>2</sub> to 16% of that observed in S<sub>1</sub>, a value significantly different (*P* < 0.01) than that obtained in S<sub>2</sub> (50%) of control nonapomorphine-treated glands. On the other hand, a low concentration (0.05 μM) of droperidol completely reversed the inhibitory effect of apomorphine on catecholamine secretion induced by nicotine and facilitated, by itself, the secretory response to nicotine (see table I). These data suggest that droperidol acts as an

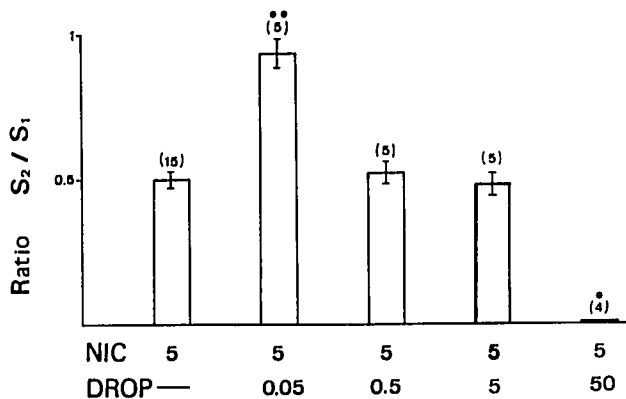


FIG. 2. Effect of low and high droperidol (DRO) concentrations on the catecholamine (CA) release evoked by nicotine (NIC). Experiments were designed as in fig. 1. Two pulses of NIC (5 μM for 2 min) 40 min apart were given to each gland. DRO (0.05, 0.5, 5, or 50 μM) was present 20 min before, during, and 6 min after NIC pulse. Ordinate shows ratios between the net CA outputs obtained during S<sub>1</sub> and S<sub>2</sub> (in presence or absence of DRO); ratios are mean ± SE of the number of experiments shown in parentheses. \**P* < 0.01; \*\**P* < 0.05. Drug concentrations have been expressed as micromolar, and they appear below the graph.

antagonist of the dopamine receptor, which modulates catecholamine release probably through a negative feedback mechanism mediated by endogenously released dopamine.<sup>8</sup>

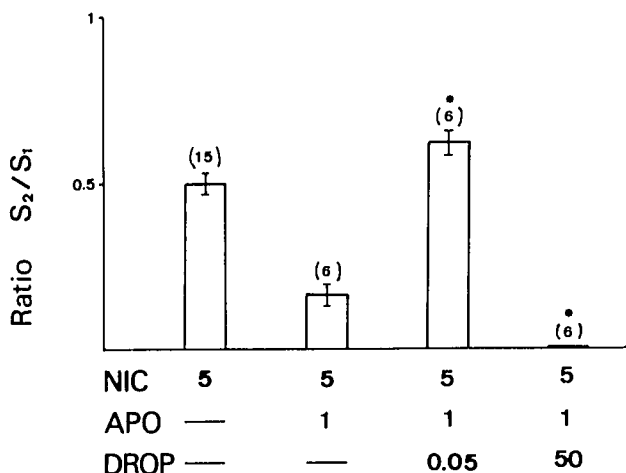


FIG. 3. Effect of different concentrations of droperidol (DRO) on the inhibition of catecholamine release produced by apomorphine (APO). A design similar to fig. 1 was carried out as follows: two pulses of nicotine (NIC for 2 min) were given to each gland 40 min apart but here, DRO (0.05 or 50 μM) was present 20 min before the second nicotine pulse and, 10 min previously to that pulse, APO was added to the perfusion medium. Both drugs were also present during S<sub>2</sub>. Ordinate shows ratios between the net CA outputs obtained during S<sub>1</sub> and S<sub>2</sub>; data are means ± se of the number of experiments shown in parentheses. \**P* < 0.01. Drug concentrations have been expressed as micromolar, and they appear below the graph.

TABLE 1. Effect of Droperidol and/or Apomorphine on the Catecholamine Release Evoked by Two Pulses of Nicotine ( $S_1$  and  $S_2$ ) in Perfused Adrenal Gland

Nicotine $S_1$ and $S_2$ (2 min)	Drugs Used		$S_2/S_1$ Ratios $\pm$ SE
	Droperidol before and during $S_2$	Apomorphine before and during $S_2$	
5 $\mu$ M (15)	—	—	0.50 $\pm$ 0.03
5 $\mu$ M (5)	0.5 $\mu$ M	—	0.94 $\pm$ 0.05*
5 $\mu$ M (6)	—	1 $\mu$ M	0.16 $\pm$ 0.03†
5 $\mu$ M (6)	0.05 $\mu$ M	1 $\mu$ M	0.62 $\pm$ 0.05‡

Results are expressed as ratios between the net catecholamine outputs obtained during  $S_1$  and  $S_2$ . Ratios are means  $\pm$  SE of the number of experiments shown in parentheses.

\*  $P < 0.05$  as compared with controls.

†  $P < 0.05$  as compared with controls.

‡  $P < 0.01$  as compared with apomorphine-treated glands.

Increased dopamine excretion has been associated with malignant pheochromocytoma,<sup>25</sup> although in most reports of benign pheochromocytomas the excretion of this amine has not been documented. However, Serrano *et al.*<sup>26</sup> also found increased dopamine excretion in some patients with benign pheochromocytoma. Additionally, Kuchel *et al.*<sup>27</sup> reported an increased plasma concentration of free and conjugated dopamine in pheochromocytoma patients. In these patients, higher than normal rates of dopamine secretion could act on adrenomedullary dopaminergic receptors to increase the "normal" inhibitory dopaminergic tone. Under these conditions, low doses of droperidol administered during neuroleptoanesthesia will remove such inhibition enhancing the release of catecholamines, and therefore is a partial explanation for hypertensive response described in some patients with pheochromocytoma.

Our results do not exclude the possibility that droperidol could also release noradrenaline from other structures, such as noradrenergic nerve endings, where a modulatory dopaminergic receptor has also been described.<sup>21,22</sup> Additionally, it has been proposed that in patients with pheochromocytoma, an increase in releasable stores of catecholamine in sympathetic nerve endings could take place due to its constant exposure to high plasma catecholamine levels<sup>28</sup>; under these conditions an effect of droperidol on dopaminergic receptors of sympathetic nerve endings could contribute to the previously described hypertensive effect that has been observed in pheochromocytoma but not in normal patients. However, the fact that dopaminergic antagonists themselves did not increase the noradrenaline release evoked by sympathetic nerve stimulation<sup>29</sup> represents the main objection for the involvement of this mechanism.

According to Desmots and Marty,<sup>4</sup> the hypertensive response to droperidol during neuroleptoanesthesia was

seldom observed when doses more than 12.5 mg were used. Sumikawa and Amakata<sup>2</sup> described severe hypertension within the first 3 min in a 13-yr-old boy with pheochromocytoma after intravenous administration of droperidol (1.25 mg) during the anesthetic induction. Although plasma concentrations of droperidol were not measured, a plasma concentration of 70–100 nM in the first 2-min period after the drug administration can be assumed. In the present study we selected a wide range of droperidol concentrations in order to explore the selectivity of droperidol as a dopaminergic adrenomedullary receptor antagonist. Our results show that when the lowest concentration (0.05  $\mu$ M) of droperidol was used, both a reversal of the inhibitory effect of apomorphine and a facilitatory effect on the catecholamine secretory response induced by nicotine were observed. This concentration is similar to those used by Steinsland and Hieble<sup>22</sup> to explore the dopaminergic antagonist action of haloperidol in the rabbit ear central arteries and is in the range of the calculated droperidol plasma concentrations. On the other hand, when higher concentrations of droperidol were used, none of the effects hitherto described were found. Instead, the highest concentration (50  $\mu$ M) of droperidol abolished the adrenal catecholaminergic secretion evoked by nicotine. The mechanism of this last effect is probably related to an interference of droperidol with calmodulin, an intracellular calcium binding protein that plays an important role in regulating many physiologic processes, including the secretory event. In fact, it has been shown that several butyrophenones, like haloperidol, have an inhibitory effect of calmodulin-dependent processes such as phosphodiesterase activity with a median inhibitory concentration ( $IC_{50}$ ) of 60  $\mu$ M,<sup>30</sup> which is in the same range of those used in our experiments.

Finally, a direct effect of the drug on tumor cells, where a nonexocytotic mechanism of release induced by droperidol has been postulated,<sup>5,7,31</sup> might also be involved. However, droperidol never induced an increase of spontaneous catecholamine release from perfused cat adrenal glands within the wide range of concentrations used. Because we have only observed an increase of exocytotic catecholamine release with droperidol in normal adrenomedullary cells, we suggest the possibility that droperidol could act directly on tumor cells only if they possess dopaminergic receptors. The fact that tumoral PC12 cells appear to retain the exocytotic mechanism,<sup>32</sup> functional nicotine receptors,<sup>9</sup> and tetrodotoxine-sensitive  $Na^+$  channels<sup>33</sup> (normally present in chromaffin cells) favors this suggestion.

In conclusion, the results of this study suggest that in addition to other mechanisms, the hypertensive response induced by low doses of droperidol during neuroleptoanesthesia in patients with pheochromocytoma could be due to the removal of an inhibitory dopaminergic mech-

anism present in the chromaffin cells, which under physiologic conditions, would limit the amount of catecholamines released from the gland or sympathetic nerves overloaded with dopamine. Such a mechanism could occur in an exaggerated manner in patients with pheochromocytoma.

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