Role for Endogenous Dopamine in Modulating Sympathetic-Adrenal Activity in Humans

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Dopamine (DA) is synthesized and secreted in central as well as peripheral nervous system and in the adrenal medulla. Neuronal DA receptors, which have been characterized as D2 receptors, mediate an inhibition of adenylate cyclase and are located prejunctionally on sympathetic nerve endings and on chromaffin cells. Their pharmacological activation causes an inhibition of in vitro and in vivo norepinephrine (NE) release from sympathetic nerve terminals and an inhibition of in vitro epinephrine (E) release from the adrenal medulla. Endogenous DA, co-secreted with the other catecholamines (CA), modulates sympathetic-adrenal discharge only during high sympathetic stimulation through an autocrine mechanism, limiting excessive sympathetic discharge. Also pheochromocytoma cells synthesize and express D2 receptors. In patients with pheochromocytoma D2 antagonists cause hypertensive crises but the mechanism mediating this effect is still unknown as well as whether endogenous DA might modulate tumoral secretion. (Hypertens Res 1995; 18 Suppl. I: S79-S86)

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Endogenous dopamine (DA) has been considered, for a long time, only a precursor in the synthesis of norepinephrine (NE) and epinephrine (E). Its biologic activity was considered almost insignificant and mainly linked to its property of weak alpha and beta adrenergic receptor agonist. The discovery of dopaminergic pathways in the brain was the first step for understanding its additional physiological role (1) which was lately confirmed by the demonstration of the presence of DA receptors (2) in the central nervous system (3) and at the periphery (4-7).

In fact, extensive pharmacological research based on the use of an increasing number of chemical compounds possessing antagonist or agonist dopaminergic activity, much more selective than DA itself, permitted to demonstrate the presence of DA receptors in the central nervous system and to divide them into two main classes, D1 and D2 (3). Further studies demonstrated the presence of DA receptors also at the periphery, in neuronal and non-neuronal tissues, and to distinguish two different receptor subtypes named DA1 and DA2 (4-7).

The recent introduction of molecular biology techniques has further improved our knowledge in the field so that it is now generally accepted (8) that: 1) Pharmacological and molecular properties of central and peripheral DA receptors are similar so that their terminology has been unified; 2) at least five subtypes of DA receptors, termed D1A (9-13), D1B (14, 15), D2 (16, 17), D3 (18), D4 (19, 20), are expressed both in the brain and at the periphery; 3) D1A and D1B receptors are coupled to the stimulation of adenylate cyclase and correspond to the classically described D1 receptors while D2, D3 and D4 receptors are coupled to the inhibition of adenylate cyclase and correspond to the classically described D2 receptors (8).

In the neuronal tissues, the DA receptors are represented by those negatively coupled to adenylate cyclase and once termed D2 or DA2.

At the periphery neuronal D2 receptors have been located in the sympathetic ganglia (21, 22), in the sympathetic nerve endings at the presynaptic level (23-30) and in the chromaffin cells of the adrenal gland (31-33).

The co-presence of DA and its receptors in the central and peripheral nervous system has prompted many studies to clarify the physiological role played by endogenous DA on the activity of the nervous system itself and on the function of those systems, mainly the cardiovascular, primarily regulated by nervous activity.

While the use of D2 agonists has permitted to clarify the biological activity mediated by D2 receptors, only the use of D2 antagonists has improved our knowledge on the role physiologically exerted by endogenous DA. In fact, pharmacological receptor blockade permits to investigate on the tonic action exerted by the endogenous ligand at the tissue level.

As a general statement, the role exerted by DA on the activity of the sympathetic adrenal system has been conducted performing in vitro and vivo ex-
While the results of the in vitro experiments generally provide definite although partial physiological data, the results of the in vivo experiments are often complicated by the contemporary direct and multiple indirect effects elicited by the dopaminergic compounds at different levels on the sympathetic-adrenal system.

**Endogenous Dopamine and Sympathetic Nerves**

In vitro studies conducted in animals of different species have definitely demonstrated that the activation of prejunctional D2 receptors located in noradrenergic nerve terminals of the central (34) as well as the peripheral nervous system (23-30, 35-39) leads to a significant decrease in NE release elicited by nerve stimulation. In vitro experiments on human cortical kidney slices have demonstrated that carmoxirole, a D2 agonist, inhibits NE release from human renal sympathetic nerves (40). In addition, it has been demonstrated that D2 agonists are able to inhibit ganglionic transmission (41, 42). All inhibitory effects are specifically counteracted by D2 antagonists (43). Nonetheless, an increase in NE release has not generally been observed after administration of D2 antagonists alone (24), casting doubts on the role played by endogenous DA in modulating sympathetic activity.

In vivo studies, performed either in animals or in man, agree on the sympatholitic effect of D2 agonists.

In man, bromocriptine (36, 44-54), apomorphine (55, 56) and co-dergocrine (57, 58) administration causes a decrease in blood pressure as well as in NE concentration in plasma (36, 45-58), urine (49) and cerebrospinal fluid (36). This effect is evoked in normal man either in resting or stimulated conditions such as upright posture (45-54), cycling activity (45), sodium depletion (49), isometric handgrip (47) and graded lower body negative pressure (53). A similar effect is observed also in pathological conditions such as essential hypertension (51, 59-63) and congestive heart failure (64).

In agreement with the in vitro studies, the in vivo inhibitory effects of D2 agonists on NE release are blocked by pretreatment with D2 antagonists such as metoclopramide and domperidone (50). Moreover, in agreement with the in vitro experiments, in vivo D2 antagonists administration to resting normal man was not able to modify cardiovascular parameters or plasma NE in our (65) as well as in other authors’ (66-69) experience, demonstrating that endogenous DA does not exert any physiological action on D2 receptors in unstimulated conditions. The question therefore arises whether endogenous DA might, in different conditions, increase at the synaptic level to concentrations high enough to stimulate prejunctional D2 receptors.

As DA is co-released with NE from sympathetic nerve endings (70-73) and as intense and/or chronic sympathetic activation increases neuronal DA release to a greater extent than NE (74), it was therefore consequential to investigate on the effects of D2 antagonists given during different degrees of sympathetic activation.

While D2 receptor blockade during mild or intermediate sympathetic activation (cold pressor, hand-grip, upright posture) did not cause any significant change in stimulated NE plasma concentrations (65), during physical exercise at 80% of maximal oxygen consumption domperidone administration caused a greater increase in plasma NE of nor-
mal volunteers (75) (Fig. 1). These results, which were confirmed by other authors (76), suggest that endogenous neuronal DA plays a physiological role in modulating NE release from noradrenergic nerve terminals during high sympathetic stimulation.

Endogenous Dopamine and Adrenal Medulla

In addition to E and NE, the adrenal medulla of several species (77), including human (78, 79), secretes DA in different amounts depending on the degree of splanchnic stimulation.

Adrenal chromaffin cells possess D2 receptors as demonstrated in several animal species (31-33, 80, 81) and in man (82) by light microscopy autoradiography, ligand binding studies and Northern blot analysis (Figs. 2 and 3).

In vitro studies using perfused bovine, cat or rabbit adrenal glands (32, 33, 83) demonstrated that D2 agonists specifically inhibit acetylcholine (Ach)-induced CA release. In vitro studies using cultured chromaffin cells (84-86) led to similar conclusions although still some controversy exists on the specificity of the inhibitory effect induced by D2 agonists (85).

Taken all together, the results of the in vitro studies demonstrate the presence of D2 receptors on adrenal chromaffin cells and their inhibitory action on CA release.

In vivo studies using D2 agonists have led, for years, to apparently conflicting results. D2 agonists have been demonstrated to decrease (47, 48) or to not alter (45, 49, 50) E plasma levels when given to normotensive subjects in supine position while an increase in plasma E was observed when D2 agonists were administered to humans kept in upright position (50).

All these results are, in our opinion, only apparently controversial. In fact, the results of studies using D2 agonists are complicated by the potent hypotensive effect induced in vivo by inhibition of NE release. This effect causes, on the adrenals, a neurally mediated-stimulus which is more potent when subjects are kept in upright position. Therefore, in these experimental conditions, the adrenal

The gland undergoes two different and opposite stimuli, a direct inhibitory one through D2 receptors and an indirect stimulatory one induced by hypotension. The net result is no change for mild degrees of baroceptor activation (supine position) or an increase for higher baroceptor mediated-sympathetic activation (upright position). In agreement with this hypothesis, Szabo et al. (43) demonstrated that in rabbits, while sodium nitroprusside induced-hypotension causes an increase in plasma E, this latter does not increase when a similar hypotension is induced by quinpirole. Moreover, when the effect of the hypotension induced-splanchnic activation is abolished, as in neurogenic hypertensive dogs, there is general (87, 88), although not universal (89), agreement that D2 agonists cause a decrease in plasma E.

We should therefore conclude that in vivo administration of D2 agonists is not a correct experimental model to investigate the dopaminergic modulation of the adrenal medulla. Less conflicting results have been obtained in vivo using D2 antagonists which, moreover, permit to clear the role played by endogenous DA on the adrenal medulla secretion.

D2 antagonist administration has been shown to cause no change in plasma E of normal subjects during unstimulated or slightly stimulated conditions (63) while a significantly higher increase in E plasma levels was observed after D2 receptor blockade in normal subjects during an intense sympathetic stimulation (75, 76) (Fig. 4).

These results suggest that in the adrenal gland, similarly to sympathetic nerve terminals, D2 receptors are stimulated by endogenous DA only when an intense splanchnic activation leads to an increase in local CA (and DA) secretion. In these conditions, adrenal DA, acting through adrenomedullary D2 receptors, causes an autocrine inhibitory modulation of CA release.

This hypothesis has been confirmed by results obtained by our group using a different in vivo experimental model. In fact, the increase in E plasma levels induced by glucagone in normal subjects resulted significantly higher after domperidone than after placebo (90) (Fig. 5). As glucagone stimulates chromaffin cells through a specific cellular receptor, these data further support the hypothesis that endogenous DA modulates medullary CA release through a DA receptor located on chromaffin cells.

Endogenous Dopamine and Tumoral Chromaffin Cells

A possible link between DA and tumoral chromaffin cells was suspected since 1976 when it was reported that metoclopramide can induce hypertensive crises in patients (91) with pheochromocytoma. The mechanism through which D2 antagonists in-
duce a secretory burst from tumoral chromaffin cells is still unknown and several hypotheses can be made: 1) D2 antagonists might act directly through D2 receptors located on pheochromocytoma cells and thus block a local autocrine inhibiting mechanism; 2) D2 antagonists might cause a CA discharge acting on prejunctional D2 receptors of sympathetic nerve terminals repleted with circulating CA.

Bromocriptine administration to patients with pheochromocytoma causes, similarly to normotensive subjects, a significant decrease in mean arterial pressure (MAP) but is not able to modify circulating CA (92) (Fig. 6). These results, while confirming that in patients with pheochromocytoma blood pressure regulation is still partly maintained by the sympathetic tone, does not clarify whether endogenous DA modulates CA release also from pheochromocytoma cells. In fact, similarly to normotensive subjects, the bromocriptine-induced decrease in MAP might cause splanchnic nerve activation and local Ach diffusion on tumoral cells. Therefore, bromocriptine might exert a dual opposite effect on tumoral cells, inhibiting them directly and activating them through local Ach secreted by the hypotension induced-splanchnic nerve activation. The net result might be no modification of CA release.

The presence of D2 receptors on pheochromocytoma cells has been demonstrated by our group in 1990 using light microscopy autoradiography (92). More recently we confirmed these data using ligand binding studies and Northern blot analysis (82) (Figs. 7 and 3). Whether D2 receptors synthesized and expressed by pheochromocytoma cells might be responsible for a modulation of CA release is still to be elucidated.

Fig. 6. Plasma norepinephrine (NE) and epinephrine (E) in 5 patients with pheochromocytoma before and after oral administration of bromocriptine (BC). Each single patient is identified by a number. In the upper right panel mean arterial pressure (MAP)(mean ± SEM) after placebo or BC is reported. (*p<0.05) (from ref. 92, with permission).

Fig. 7. a) Autoradiogram of standardized dot blot analysis performed on 4 pheochromocytomas (PHEO) and rat brain (B). Sixty micrograms of total RNA from each PHEO and 10 µg of total RNA from B were blotted to a positively charged nylon membrane and hybridized to a 32P labelled human D2 receptor cDNA. b) 18S ribosomal RNA stained with ethidium bromide.

References


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