Dopaminergic control of angiotensin II-induced vasopressin secretion in vitro

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Rossi, Noreen F. Dopaminergic control of angiotensin II-induced vasopressin secretion in vitro. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E687–E693, 1998.—Because dopamine influences arginine vasopressin (AVP) release, the present studies were designed to ascertain the dopamine receptor subtype that potentiates angiotensin II-induced AVP secretion in cultured hypothalalo-neurohypophysial explants. Dopamine (a nonspecific D1/D2 agonist), apomorphine (a D2 > D1 agonist), and SKF-38393 (a selective D1 agonist) dose dependently increased AVP secretion. Maximal AVP release was observed with 5 µM dopamine, 307 ± 66%· explant⁻¹· h⁻¹, 1 µM SKF-38393, 369 ± 41%· explant⁻¹· h⁻¹, and 0.1 µM apomorphine, 374 ± 67%· explant⁻¹· h⁻¹. Selective D1 antagonism with 1 µM SCH-23390 blocked AVP secretion to values no different from basal. Dopamine (D1 agonist), phenoxybenzamine (nonspecific adrenergic antagonist), and prazosin (α1-agonist) failed to prevent release. D1 antagonism also prevented AVP secretion to 1 µM angiotensin II [angiotensin II, 422 ± 87%· explant⁻¹· h⁻¹ vs. angiotensin II plus SCH-23390, 169 ± 28%· explant⁻¹· h⁻¹ (P < 0.05), but D1 and α1-adrenergic blockade did not. In contrast, AT1 receptor inhibition with 0.5 µM losartan blocked angiotensin II- but not dopamine-induced AVP release. AT1 antagonism had no effect. Although subthreshold doses of the agonists did not increase AVP secretion (0.05 µM dopamine, 133 ± 44%· explant⁻¹· h⁻¹, 0.01 µM SKF-38393, 116 ± 26%· explant⁻¹· h⁻¹, and 0.001 µM angiotensin II, 104 ± 29%· explant⁻¹· h⁻¹), the combination of dopamine and angiotensin II provoked a significant rise in AVP [420 ± 83%· explant⁻¹· h⁻¹ (P < 0.01)]. Similar results were observed with SKF-38393 and angiotensin II, and the AVP response was blocked to basal levels by either D1 or AT1 antagonism. These findings support a role for D1 receptor activation to increase AVP release and mediate angiotensin II-induced AVP release within the hypothalalo-neurohypophysial system. The data also suggest that the combined subthreshold stimulation of receptors that use distinct intracellular pathways can prompt substantial AVP release.

angiotensin receptors; dopamine receptors; hypothalalo-neurohypophysial system; supraoptic nucleus

METHODS

Experiments were performed on male Long-Evans rats weighing 125–150 g. The rats were housed at constant temperature with a 12:12 h light-dark cycle. They were given free access to water and standard rodent chow. All procedures were reviewed and approved by the institutional Animal Investigation Committee and were in compliance with the National Institutes of Health guidelines.

Dissection and culture of explants. Explants of the hypothalalo-neurohypophysial system were dissected as previously described (30, 34). Each explant contained supraoptic nuclei with intact axonal projections to the neural lobe. The

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ergic antagonist, 1 µM phenoxybenzamine; antagonist, 1.5 µM domperidone, and the nonselective adren-
cortic nuclei and subfornical organ were absent. Each explant was placed into a separate incubation well (Falcon, Oxnard, CA) and was supported ventral side down by Teflon mesh (Spectrum, Los Angeles, CA). The culture me-
ium was Ham's F-12 nutrient mixture with 5.5 mM dext-
ose, 100 U/ml penicillin, and 100 µg/ml streptomycin (Life
Technologies, Grand Island, NY) fortified with 20% fetal
bovine serum (Hydnone, Logan, UT). Medium was incubated at 37°C under a humidified atmosphere of 95% O₂–5% CO₂. Medium was changed at 24-h intervals.

Sampling procedures. Protocols were performed 48 h after
dissection and follow the sampling techniques reported ear-
erly (34). All experiments were performed in the presence of the peptidase inhibitor bacitracin, 0.05 mg/ml to prevent degradation of released AVP (30). Immediately before the protocol, ascorbic acid was added to each well to bring the final concentrate to 10 µM to retard the oxidation of dopamine and its analogs.

During the basal period, AVP release rate was ascertained after 1 h of exposure to standard medium. This was followed immediately by a 1-h test period. All test agent(s) were dissolved in medium containing 10 µM ascorbic acid. Vehicle controls were performed whenever applicable. Samples were obtained for determination of AVP release rates and degradation during both basal and test periods. AVP release rates were corrected for volume of medium and degradation (34). Samples for AVP radioimmunoassay were frozen and stored at −70°C. Osmolality was determined on the remaining medium.

Protocols. The following protocols were performed: 1) dose-
response curves for 0.01–10 µM dopamine (nonselective D₁/D₂ agonist), 0.01–10 µM SKF-38393 (selective D₁ agonist), and 0.01 nM to 1 µM apomorphine (D₂ > D₁ agonist); 2) nonselective dopaminergic antagonism of dopamine-induced AVP release by 1 µM haloperidol; 3) antagonism of maximally stimulating doses of each agonist (1 µM angiotensin II, 5 µM dopamine, 1 µM SKF-38393, and 0.1 µM apomorphine) using the nonselective D₁ antagonist, 1 µM SCH-23390, or the D₂ antagonist, 1.5 µM domperidone, and the nonselective aden-
ergic antagonist, 1 µM phenoxybenzamine; 4) angiotensin receptor blockade of each agonist with the nonselective antagonist 1 µM saralasin, the AT₁ antagonist, 0.5 µM losartan, or the AT₂ antagonist, 0.5 µM CGP-42112A; 5) antagonism of 1 µM SKF-38393 or 1 µM angiotensin II with 1 µM SCH-23390 or the selective 1 µM α₁-prazosin alone or in combination; 6) the combination of submaximally stimulating concentrations of 0.05 µM dopamine or 0.01 µM SKF-38393 with a subthreshold dose of 0.01 µM angiotensin II; and 7) dopaminergic or angiotensinergic receptor inhibition of the combined effect of submaximal angiotensin II and dopamine agonists. Doses of the antagonists were chosen based on reported values for inhibition constants.

Analytic methods. AVP content of the medium was mea-
sured by radioimmunoassay using methods reported earlier from our laboratory (30). All standards and samples were assayed in duplicate; samples were diluted 1:100 with buffer and assayed directly. Standards were prepared with purified AVP (Ferring, Malmo, Sweden). The tracer was [¹²⁵I]iodotyro-
syl AVP (Amersham, Arlington Heights, IL). Anti-AVP serum
no. 2849 was used at a final dilution of 1:3.6 × 10⁵. Assay
buffer contained 0.15 M sodium phosphate, 0.01 M sodium
EDTA, 0.1 g/100 ml sodium azide, and 0.1 g/100 ml bovine
serum albumin (ICN-Miles, Irvine, CA) at pH 7.4. Assay
sensitivity and cross-reactivities have been reported pre-
viously (30). Medium osmolality was measured by freezing point depres-
sion (Precision Systems 5004, Sudbury, MA).

Domperidone was obtained from Janssen Pharmaceutica (Piscataway, NJ). Losartan was provided by Merck (Rahway, NJ). Dopamine, SKF-38393, SCH-23390, CGP-42112A, and phenoxybenzamine were obtained from Research Biochemicals (Natick, MA). Unless otherwise stated, all other chemi-
cals were obtained from Sigma (St. Louis, MO).

Statistics. All comparisons between basal and test period release rates were performed on the absolute release rates. Because basal AVP release rates varied among the groups of explants, the release of AVP during the test hour was normalized as the percentage of AVP released during the preceding basal hour for the same explant. Each explant acted as its own control.

Differences between basal and test hour release of AVP in
the same explants were compared using the paired t-test. Analyses among the test period secretory rates were performed on the normalized values. Comparisons between two test periods from separate explants were performed by Stu-
dent’s t-test. When multiple comparisons were made, signifi-
cant differences were assessed by analysis of variance for repeated measures and the Tukey-Kramer test for multiple comparisons. All data are expressed as means ± SE. P < 0.05 was taken as significant.

RESULTS

Overall basal AVP release rate at 48 h was 79 ± 2
pg· explant⁻¹· h⁻¹ (n = 418). Basal medium osmolality was
305 ± 1 mosmol/kgH₂O and did not change by the end of the test period (307 ± 2 mosmol/kgH₂O). AVP release did not vary during time control experiments with standard medium administered during both basal and test periods [basal, 100 ± 25 vs. test, 115 ± 6% explant⁻¹· h⁻¹ (P > 0.05, n = 7)].

Dose-response curves for the dopamine agonists are shown in Fig. 1. AVP release was significantly increased by concentrations of dopamine or SKF-38393 >1 µM or apomorphine >10 nM. Nonselective dopaminergic receptor blockade with haloperidol inhibited dopamine-induced AVP secretory activity (Fig. 2). The vehicle for haloperidol, 0.0038% methanol, did not alter AVP release [basal (no methanol) 100 ± 35 vs. test

![Fig. 1. Test period arginine vasopressin (AVP) secretory rate by
explants in response to increasing doses of dopamine (○; n = 6, 6, 6,
and 7), SKF-38393 (●; n = 6, 6, 6, 6, 6, 6, and 6), and apomorphine (●;
n = 6, 7, 8, 8, 6, and 6). Values are means ± SE. *P < 0.05 vs. basal
release rate by paired t-test. (Agonist), agonist concentration.]
methylol vehicle) 128 ± 28%·explant⁻¹·h⁻¹. The dose–response relationship to dopamine was unchanged by the presence of methanol [compare Figs. 1 (without methanol) and 2 (with methanol)]. Haloperidol alone did not alter basal AVP secretory rate [basal, 100 ± 31 vs. haloperidol, 86 ± 20%·explant⁻¹·h⁻¹ (n = 8; P > 0.05)].

Figure 3 shows the effect of the selective D₁ and D₂ dopamine receptor antagonists and the nonselective adrenergic blocker on dopamine-induced AVP release. Significant inhibition occurred only with SCH-23390. The D₁ dopaminergic blocker also blocked AVP release by maximally stimulating doses of SKF-38393 or apomorphine, as depicted in Table 1. Domperidone also failed to inhibit AVP release by 1 µM SKF-38393 (369 ± 41) vs. SKF-38393 with domperidone [397 ± 72%·explant⁻¹·h⁻¹ (P > 0.05, n = 6 and 6, respectively)] or 0.1 µM apomorphine (392 ± 67) vs. apomorphine with domperidone [309 ± 33%·explant⁻¹·h⁻¹ (P > 0.05, n = 6 and 7, respectively)]. SCH-23390 alone did not change AVP release [basal, 100 ± 21 vs. SCH-23390, 165 ± 25%·explant⁻¹·h⁻¹ (P > 0.05, n = 7)] nor did domperidone [basal, 100 ± 10 vs. domperidone, 104 ± 15%·explant⁻¹·h⁻¹ (P > 0.05, n = 6)].

D₂ antagonism prevented the AVP secretory response to a maximally stimulating dose of angiotensin II as well as the response to the D₂ agonists. D₂ antagonism did not alter the effect of any of the agonists (Fig. 4). Figure 5 shows that α₁-adrenergic blockade with prazosin did not inhibit AVP release to either SKF-38393 or angiotensin II. No significant additional inhibition occurred with the combined D₁ dopaminergic and α₁-adrenergic antagonists. In contrast, AT₁ receptor blockade inhibited only angiotensin II-stimulated but not dopamine- or SKF-38393-induced AVP release. AT₂ receptor inhibition did not prevent AVP secretion (Fig. 6). Given alone, neither losartan nor CGP-42112A changed basal AVP secretory rate [82 ± 27 and 60 ± 19%·explant⁻¹·h⁻¹, respectively (P > 0.05 vs. basal)].

A submaximal concentration of either dopamine or SKF-38393 in combination with a subthreshold dose of angiotensin II prompted a significant release of AVP. Stimulation by the combined agonists was blocked by both D₁ and AT₁ inhibitors as well as nonselective angiotensin receptor antagonism (Table 2).

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**Table 1. Selective D₁ dopamine receptor antagonism of AVP release in response to dopamine agonists**

<table>
<thead>
<tr>
<th></th>
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<th>Basal</th>
<th>Test</th>
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<tr>
<td>DA</td>
<td>8</td>
<td>100 ± 30</td>
<td>307 ± 66*</td>
</tr>
<tr>
<td>SKF</td>
<td>8</td>
<td>100 ± 30</td>
<td>369 ± 41*</td>
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<tr>
<td>APO</td>
<td>8</td>
<td>100 ± 43</td>
<td>374 ± 67*</td>
</tr>
<tr>
<td>DA + SCH</td>
<td>8</td>
<td>100 ± 21</td>
<td>115 ± 22†</td>
</tr>
<tr>
<td>SKF + SCH</td>
<td>8</td>
<td>100 ± 18</td>
<td>148 ± 38‡</td>
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<tr>
<td>APO + SCH</td>
<td>8</td>
<td>100 ± 31</td>
<td>92 ± 39‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of observations. DA, 5 µM dopamine; SKF, 1 µM SKF-38393; APO, 0.1 µM apomorphine; SCH, 1 µM SCH-23390. *P < 0.01 vs. basal values by paired t-test. †P < 0.05 and ‡P < 0.01, vs. the respective agonist alone by ANOVA.

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Fig. 2. AVP release by explants in response to dopamine (○; n = 6, 8, 8, and 7) or dopamine in the presence of 1 µM haloperidol (●; n = 8, 6, 7, and 7). Methanol, the vehicle for haloperidol, was present during the test period in all groups. Data are means ± SE. *P < 0.05 vs. basal by paired t-test. ^P < 0.05 vs. same concentration of dopamine ([Dopamine]) alone by ANOVA.

Fig. 3. Antagonism of dopamine (DA, 5 µM, n = 6)-induced AVP release with SCH-23390 (1 µM SCH; n = 7), domperidone (1.5 µM DMD, n = 6), or phenoxybenzamine (1 µM PBZ, n = 6). Basal (open bars) and test period (filled bars) release rates are shown. Data are means ± SE. *P < 0.05 vs. preceding basal period by paired t-test. ^P < 0.025 vs. DA test period by ANOVA.

Fig. 4. Selective dopamine receptor antagonist with 1 µM SCH-23390 (SCH) or with 1.5 µM domperidone (DMD) of AVP release induced by 5 µM dopamine (filled bars), 1 µM SKF-38393 (open bars), or 1 µM angiotensin II (crosshatched bars). Values are means ± SE; n = 6 for each of the nine groups except for angiotensin II alone where n = 8. *P < 0.05 vs. basal release rate by paired t-test. †P < 0.05 vs. agonist alone by ANOVA.
well as direct injections of dopamine into the supraoptic nucleus of rats (3, 32) by Sladek and colleagues (18, 32) by perifused explants and in cultured neurons from supraoptic nuclei where the confounding effects of osmolality, extracellular volume, systemic arterial pressure, or anesthesia could be controlled or eliminated, dopamine stimulated AVP release when hormone levels were already elevated, but when AVP levels were low, as in water-loaded rats (7, 15, 17, 20), but some investigators observed suppression or no change in AVP secretion with dopamine (4, 39). The variability of the response to dopamine was highly dependent on the initial activity of the neurohypophysial system. Dopamine had little, if any, effect on AVP release when hormone levels were already elevated, but when AVP levels were low, as in water-loaded rats, dopamine increased AVP (7, 31). In our explants, where the confounding effects of osmolality, extracellular volume, systemic arterial pressure, or anesthesia could be controlled or eliminated, dopamine stimulated AVP secretion.

Our findings clearly implicate a D₁ receptor as responsible for the increase in AVP. Earlier studies were compelled to rely on nonselective ligands, and stimulation of other catecholaminergic receptors, such as α-adrenergic receptors, often was not excluded (7, 15, 17, 25, 26). The present studies extend observations made in perfused explants and in cultured neurons from supraoptic nucleus by Sladek and colleagues (18, 32) by showing the concentration dependency and the D₁ selectivity of the AVP stimulatory response. Dopamine fibers terminate synaptically on magnocellular neuron cell bodies within the supraoptic nucleus (3, 6). These cells express D₃A mRNA and exhibit D₁ receptors on the cell membrane (8, 18). Dopaminergic fibers also project from the arcuate nucleus to the neurointermediate lobe and end in close proximity to the AVP neurosecretory endings (3, 17, 23). Experiments in these explants do not distinguish between these sites of action. D₁ receptors at either or both of these loci could potentially have been activated. Data exist showing that, at least at the level of the neural lobe, D₁ agonists facilitate electrically evoked AVP release (25, 26).

| Table 2. Combined effects of submaximally stimulating doses of dopamine or SKF-38393 with angiotensin II |
|-------------------------------------------------|---------------------|----------------|---|
| n         | Basal | Test  | P Value |
| DA        | 7     | 100±41 | 133±44 | 0.05 |
| SKF       | 7     | 100±30 | 116±26 | 0.05 |
| ANG II    | 8     | 100±34 | 104±29 | 0.05 |
| DA + ANG II | 6    | 100±43 | 420±83 | <0.01 |
| DA + ANG II + LOS | 7  | 100±29 | 52±26* | 0.05 |
| DA + ANG II + CGP | 8 | 100±33 | 426±72 | <0.002 |
| DA + ANG II + SAR | 7  | 100±29 | 147±52* | 0.05 |
| DA + ANG II + SCH | 6  | 100±48 | 99±36* | 0.05 |
| SKF + ANG II | 7  | 100±35 | 307±56 | <0.01 |
| SKF + ANG II + LOS | 6  | 100±44 | 112±48† | 0.05 |
| SKF + ANG II + CGP | 8 | 100±46 | 314±43 | <0.005 |
| SKF + ANG II + SAR | 7  | 100±37 | 102±30* | 0.05 |
| SKF + ANG II + SCH | 7  | 100±24 | 84±195 | 0.05 |

Values are means ± SE; n, number of observations. DA, 0.05 µM dopamine; SKF, 0.01 µM SKF-38393; ANG II, 1 nM angiotensin II; LOS, 0.5 µM losartan; CGP, 0.5 µM CGP-42112A; SAR, 1 µM saralasin; SCH, 1 µM SCH-23390. P values for comparisons between basal and test periods by paired t-test are shown. *P < 0.05 vs. DA + angiotensin II; †P < 0.05 vs. DA + angiotensin II; ‡P < 0.01 vs. SKF + angiotensin II; §P < 0.05 vs. SKF + angiotensin II by ANOVA.
Numerous studies have shown that angiotensin II stimulates AVP secretion (30, 31, 33). AT$_1$ receptors identified within hypothalamic loci have been implicated in AVP release (19, 22, 24). Qadri et al. (24) have shown that microinjection of losartan into supraoptic nuclei reduced AVP release after angiotensin II was administered intracerebroventriculally, but AVP secretion was still twofold higher than baseline levels. In the present experiments, losartan inhibited AVP secretion by the explants to basal levels. A plausible explanation for this disparity is that the explants do not contain the subfornical organ or the paraventricular nuclei. Thus angiotensin II in the medium was only able to act upon AT$_1$ receptors on the magnocellular cell body itself or on neuronal pathways within the explant (33). Angiotensin II administered in vivo may have acted on the circumventricular organs to elicit AVP release not only via well-characterized angiotensinergic pathways (12) but also via $\alpha_2$-adrenergic pathways (37).

In dogs, nonselective dopamine receptor antagonism prevented AVP release in response to intravenous angiotensin II (2). Our data indicate that a D$_1$ dopamine receptor mechanism is responsible. Clearly, when the D$_1$ antagonist was present, AVP secretion in response to a maximally stimulating concentration of angiotensin II was reduced by 84% to a level that did not differ from the basal release rate. Within the substantia nigra, AT$_1$ receptor activation potentiated dopamine release from nerve terminals (11). A similar scheme in the hypothalmo-neurohypophysial system would result in angiotensin II inducing dopamine release from nerve terminals within the explant. Dopamine, in turn, would act upon D$_1$ receptors on the magnocellular cell body, along the axon, or on the neural lobe. In the absence of D$_1$ receptor activation, as seen with D$_1$ receptor antagonism, angiotensin II stimulation of the magnocellular neuron fails to release AVP. This does not imply that a dopaminergic neuron or a D$_1$ receptor mediates angiotensin II actions. These findings do provide evidence, however, that D$_1$ input at either the cell body or the axon of the magnocellular neuron is required for AT$_1$ activation to stimulate AVP release (Fig. 7).

The involvement of an $\alpha_2$-adrenergic pathway in supraoptic nucleus mediating the angiotensin II effect has also been suggested; however, AVP levels were reduced only by 50% when prazosin was microinjected bilaterally (24). The residual AVP concentrations after $\alpha_2$-adrenergic blockade remained at least threefold higher than baseline levels reported using the same paradigm. Veltmar et al. (37) have shown that the effect of $\alpha_1$- and $\alpha_2$-adrenergic antagonism was more pronounced on AVP release prompted by angiotensin II within the paraventricular nuclei, which are not contained within the hypothalmo-neurohypophysial explants. In fact, $\alpha_2$-adrenergic blockade did not change AVP release to angiotensin II by the explants. The small additional decrement that prazosin elicited when used in conjunction with SCH-23390 was not significant. Thus our findings suggest that dopamine plays a major role in mediating AVP release stimulated by angiotensin II acting at the supraoptic nucleus.

The D$_1$ dopamine receptor is linked to adenylyl cyclase (13, 16). Several investigators have also observed that D$_1$ dopamine receptor activation increases cytosolic free calcium (9, 36). Although influx of extracellular calcium is not required, cytoplasmic calcium concentration is an important determinant of the response to dopamine in vasopressinergic magnocellular neurons (29). In contrast, AT$_1$ receptor signaling occurs by activation of the phosphoinositide cascade in the central nervous system (10, 21, 35). Because coadministration of subthreshold concentrations of D$_1$ agonists and angiotensin II potentiated AVP secretion, it appears that simultaneous occupation of D$_1$ and AT$_1$ receptors at low levels on the magnocellular neurons leads to sufficient activation of these separate pathways so as to elicit a rise in intracellular calcium sufficient to provoke a large secretory response. Such an interaction between dopamine and angiotensin II to augment intracellular calcium could quite conceivably occur at the nerve terminal. If the interaction is at the level of the cell body, the mechanisms for modulating the propagation of action potentials to the neural lobe may be more varied and complex. For example, it could involve the participation of excitatory and/or inhibitory amino acid receptors via activation of protein kinase A or protein kinase C by dopamine and angiotensin II. Finally, an action at the level of the soma does not necessarily exclude an action at the level of the axon or nerve terminal.

Taken together, the present data provide evidence that D$_1$ dopamine receptor activation concentration...
dependently increases AVP secretion. The findings further support the concept that activation of a D<sub>1</sub> dopaminergic pathway is required for angiotensin II-induced AVP release within the hypothalamo-neurohypophysial explant. We can speculate that the need for dual inputs would allow for integration and fine tuning of AVP in the whole organism. Finally, the potentiation of AVP secretion by combined subthreshold D<sub>1</sub> and AT<sub>1</sub> agonism is consistent with sufficient activation of apparently distinct intracellular pathways to increase intracellular calcium to a level that prompts secretion. Additional studies are warranted to localize the site of dopaminergic modulation of the angiotensin II response more precisely, such as magnocellular cell body versus neurointermediate lobe.

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REFERENCES


