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Research Report

# Interruption of central neuronal pathway of imidazoline $I_1$ receptor mediates the hypertensive effect of cyclosporine in rats

*Mahmoud M. El-Mas*, Amal G. Omar, Mai M. Helmy, Mahmoud M. Mohy El-Din*

*Department of Pharmacology, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt*

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ABSTRACT

Increased central sympathetic outflow secondary to afferent sympathetic excitation has been implicated in the hypertensive effect of the immunosuppressant drug cyclosporine (CSA). The present study investigated the roles of central $\alpha_2$-adrenoceptors and $I_1$-imidazoline receptors in modulating the hypertensive action of CSA. The blood pressure (BP) response to CSA in conscious rats was assessed in the absence and presence of peripherally or centrally acting sympatholytic drugs. Also, the effect of selective pharmacologic blockade of $\alpha_2$ or $I_1$ receptors by yohimbine and efaroxan, respectively, on the pressor response to CSA was evaluated. CSA (20 mg/kg i.v.) produced a rapid increase in BP that peaked (25±4 mm Hg) after approximately 4 min and continued for the 45 min study duration. Ganglionic (hexamethonium 20 mg/kg) or $\alpha_1$-adrenoceptor (prazosin 1 mg/kg) blockade reduced the pressor effect of CSA. Pressor responses to phenylephrine ($\alpha_1$-adrenoceptor agonist) were not affected by CSA, thereby eliminating a possible role for alterations of vascular $\alpha_1$-adrenoceptor responsiveness in CSA hypertension. CSA hypertension was attenuated in rats pretreated intravenously with drugs that reduce central sympathetic tone including clonidine (mixed $\alpha_2/I_1$-receptor agonist, 30 μg/kg) or moxonidine (selective $I_1$-receptor agonist, 100 μg/kg) in contrast to no effect for guanabenz (selective $\alpha_2$-receptor agonist, 30 μg/kg). Intracisternal (i.c.) administration of moxonidine also reduced CSA hypertension. Selective blockade of central $I_1$ (efaroxan, 0.15 μg/rat, i.c.) but not $\alpha_2$ (yohimbine, 25 μg/5 μl/rat, i.c.) receptors abolished the hypertensive response to CSA. Together, these findings highlight that CSA elicits its hypertensive effect via disruption of central sympathoinhibitory pathways which include $I_1$-imidazoline receptors.



## 1. Introduction

Cyclosporine is an immunosuppressant drug that inhibits the immune system by suppressing T-helper cell activation via inhibition of calcineurin, a critical intracellular enzyme of the immune response cascade (Mason, 1990). CSA is used in organ transplantation to prolong the survival of allogenic grafts and in the treatment of autoimmune diseases (Cohen et al., 1984). However, the clinical use of cyclosporine is often associated with serious cardiovascular disorders such as hypertension.

* *Corresponding author*. Fax: +203 487 3273.
E-mail address: mahelm@hotmail.com (M.M. El-Mas).
*Abbreviations:* CSA, cyclosporine; BP, blood pressure; MAP, mean arterial pressure; i.c., intracisternal

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Approximately 80% of patients treated with CSA after kidney transplantation develop hypertension (Ponticelli et al., 1993). Also, CSA causes hypertension in 70% of liver transplant patients and almost 100% of cardiac transplant recipients (Diaz et al., 1989; Eid et al., 1989). Endothelium dysfunction (Oriji, 2003), arterial baroreceptor impairment (El-Mas et al., 2002a,b), and activation of endothelin (Gardiner et al., 2004) and renin–angiotensin (Nishiyama et al., 2003) systems are possible mechanisms of the pressor response to CSA. Convincing evidence is also available that sympathoexcitation plays an important role in CSA-evoked hypertension. CSA increases: (i) renal and lumbar sympathetic nerve activity (Morgan et al., 1991; Zhang and Victor, 2000), (ii) sympathetic discharge to leg skeletal muscle (Scherrer et al., 1990), and (iii) tyrosine hydroxylase activity and mRNA expression in the adrenal medulla (Shimizu et al., 2001). The attenuation of CSA-induced increases in vascular resistance and BP after peripheral (hexamethonium) or central (clonidine) sympathetic blockade provides pharmacological evidence for the involvement of sympathetic neural activity in the hypertensive action of CSA (Morgan et al., 1991).

Reported findings highlight an important role for central neurons in the sympathoexcitatory and hypertensive actions of CSA. Chiu et al. (1992) reported that the pressor effect of CSA is blunted in pithed rats. Others have shown that the increase caused by CSA in efferent sympathetic discharge follows the CSA-induced localized increases in renal and subdiaphragmatic afferents, which project centrally via the vagal and dorsal root ganglia (Moss et al., 1985; Lyson et al., 1994; Zhang and Victor, 2000). Surgical interruption of these afferent inputs produced by subdiaphragmatic vagotomy or dorsal rhizotomy eliminates the increases in subdiaphragmatic discharges and hypertension elicited by CSA (Lyson et al., 1994). Based on these findings, the postulate has been made that afferent excitation is the primary neuronal event triggered by CSA, which results in a reflex activation of efferent sympathetic activity and subsequently hypertension (Moss et al., 1985; Lyson et al., 1994; Zhang and Victor, 2000). With that said, a direct interaction of CSA with central neurons is unlikely because: (i) systemic CSA has a limited capacity to diffuse to brain tissues (Niederberger et al., 1983), and (ii) CSA causes no changes in BP or sympathetic activity when administered centrally (Lyson et al., 1994).

Despite the established intermediary role of central neurons in the pressor and sympathoexcitatory actions of CSA, the precise central pathway that modulates hemodynamic effects of CSA remains poorly understood. A possible role for medullary cardiovascular neurons in CSA hypertension is supported by the observation that the centrally acting sympatholytic drug clonidine reduces the hypertensive response to CSA (Morgan et al., 1991). This latter study, however, suffered three limitations. First, clonidine was administered systemically, raising the possibility that peripheral effects of clonidine (Langer, 1981) might also be involved in its interaction with CSA. Second, experiments were undertaken in anesthetized rats (Morgan et al., 1991). Anesthesia is known to alter neural control of sympathetic and cardiovascular functions (El-Mas et al., 1994). Third, given that clonidine has a relatively low $I_1/\alpha_2$ receptor affinity ratio (Ernsberger et al., 1993) and its hypotensive action is mediated via the activation of both receptor sites (Timmermans and Van Zwieten, 1982; Bousquet et al., 1984, 1992), the study by Morgan et al. (1991) provided no information regarding whether $I_1$ and $\alpha_2$ receptors differentially contribute to the cardiovascular actions of CSA. These problems were circumvented in the current study, which evaluated the hemodynamic effects of CSA in conscious freely moving rats pretreated systemically with centrally acting sympatholytic drugs that exhibit selective agonistic activity at $\alpha_2$ and $I_1$ receptor sites, namely moxonidine (selective $I_1$-receptor agonist), guanabenz (selective $\alpha_2$-receptor agonist), and clonidine (mixed $\alpha_2/I_1$-receptor agonist) (Bousquet et al., 1992; Ernsberger et al., 1993). Because preliminary results demonstrated a preferential involvement of $I_1$-receptors in CSA hypertension, more studies were conducted to: (i) provide a direct evidence for the involvement of central $I_1$-receptor pathway in the interaction by determining the cardiovascular actions of i.v. CSA in rats pretreated intracisternally (i.c.) with moxonidine, and (ii) investigate the effect of selective blockade of central $I_1$ or $\alpha_2$ sites by efaroxan and yohimbine, respectively, on CSA hypertension. Notably, reported studies have shown that central $\alpha_2$ and $I_1$ receptors modulate bulbospinal sympathetic discharges from the brainstem rostral ventrolateral neurons (Chalmers and Pilowsky, 1991; Bousquet et al., 1992). Further, the reduction in central sympathetic outflow caused by the activation of brainstem $\alpha_2/I_1$ receptors is the principal mechanism that underlies the hypotensive effect of clonidine and related centrally acting antihypertensive agent (Timmermans and Van Zwieten, 1982; Bousquet et al., 1984, 1992).

**Table 1 – Baseline values of mean arterial pressure (MAP, mm Hg) and heart rate (HR, beats/min)**

| Group | N | MAP | HR |
|---|---|---|---|
| Saline (i.v.) + cremophor | 9 | 107 ± 7 | 392 ± 27 |
| Saline (i.v.) + CSA | 8 | 105 ± 9 | 390 ± 28 |
| Hexamethonium + cremophor | 6 | 112 ± 3 | 426 ± 26 |
| Hexamethonium + CSA | 7 | 113 ± 3 | 413 ± 18 |
| Prazosin + cremophor | 6 | 122 ± 7 | 410 ± 22 |
| Prazosin + CSA | 6 | 109 ± 3 | 416 ± 21 |
| Phenylephrine (cremophor) | 7 | 117 ± 6 | 416 ± 32 |
| Phenylephrine (CSA) | 7 | 104 ± 5 | 420 ± 21 |
| Moxonidine (i.v.) + cremophor | 6 | 124 ± 9 | 401 ± 15 |
| Moxonidine (i.v.) + CSA | 6 | 106 ± 9 | 354 ± 17 |
| Clonidine + cremophor | 7 | 112 ± 5 | 400 ± 32 |
| Clonidine + CSA | 8 | 103 ± 7 | 402 ± 26 |
| Guanabenz + cremophor | 7 | 116 ± 5 | 410 ± 19 |
| Guanabenz + CSA | 8 | 110 ± 4 | 376 ± 20 |
| Saline (i.c.) + cremophor | 6 | 111 ± 3 | 394 ± 25 |
| Saline (i.c.) + CSA | 7 | 114 ± 7 | 385 ± 23 |
| Moxonidine (i.c.) + cremophor | 5 | 110 ± 3 | 416 ± 11 |
| Moxonidine (i.c.) + CSA | 6 | 102 ± 3 | 386 ± 24 |
| Saline(i.c.) + saline (i.c.) | 6 | 111 ± 3 | 394 ± 25 |
| Efaroxan (i.c.) + moxonidine (i.c.) | 6 | 103 ± 4 | 395 ± 23 |
| Yohimbine (i.c.) + moxonidine (i.c.) | 6 | 105 ± 10 | 400 ± 21 |
| Efaroxan (i.c.) + cremophor (i.v.) | 6 | 102 ± 4 | 401 ± 24 |
| Efaroxan (i.c.) + CSA (i.v.) | 6 | 112 ± 4 | 381 ± 11 |
| Yohimbine (i.c.) + cremophor (i.v.) | 7 | 108 ± 7 | 377 ± 16 |
| Yohimbine (i.c.) + CSA (i.v.) | 7 | 120 ± 3 | 373 ± 10 |

Values are means ± SEM.