J. Pharm. Pharmacol. 1994, 46: 397–399
Received January 26, 1993
Accepted August 31, 1993

Evidence for a dopaminergic involvement in the renal action of centrally administered JA116a, a novel compound with possible dopaminergic activity, in rats

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Abstract—Intracerebroventricular (i.c.v.) administration of JA116a, induces an increase in urinary volume and sodium excretion in conscious male hydrated rats. The involvement of brain dopaminergic neurons in the JA116a renal action was investigated. Diuretic and natriuretic action of JA116a was blocked by haloperidol pretreatment. The renal effect was prevented by selective dopaminergic neuron, denervation by i.c.v. administration of 6-hydroxydopamine in combination with desmethylimipramine. Our results suggest that JA116a acts centrally, at least in part, via an interaction with endogenous dopamine neurons.

Structure-activity relationship studies of many classes of dopamine receptor agonists allow some generalizations regarding dopaminergic activity. The dopaminergic pharmacophore is generally considered to consist of an aromatic ring bearing a *m*-hydroxy group or a pyrrol, pyrazolone or 2-aminothiazole ring mimicking these two functions, separated from a basic amino group to give a similar *N*-*m*-hydroxy distance to dopamine (Hacksell et al 1981). We recently synthesized JA116a, a rigid nonhydroxylated amino-indane (Fig. 1).

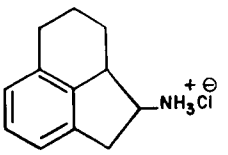

FIG. 1. Chemical structure of JA116a.

We have shown that this novel compound with possible dopaminergic activity, when administered centrally induces diuresis and natriuresis in a dose-dependent manner (Orfila et al 1992). Since JA116a contains a dopaminergic pharmacophore and its action is similar to that of dopamine, we were prompted to evaluate the possible involvement of the brain dopaminergic system in the diuretic and natriuretic action produced by centrally administered JA116a. For this purpose, we assessed the renal effects of JA116a after dopamine receptor blockade by haloperidol. In addition, we studied the ability of intracerebroventricular administration of the catecholaminergic neurotoxin, 6-hydroxydopamine, after desmethylimipramine blockade of neuronal catecholamine uptake for noradrenaline, to cause a selective central dopaminergic denervation. This treatment would result in a diminished dopaminergic tone in the median eminence and pituitary gland (Annunziato 1979).

Materials and methods

Drugs. JA116a is a compound with a primary amine tricyclic structure and phenylethylamine fragment, synthesized at the Laboratories of Medicinal Chemistry, Faculty of Pharmacy, Universidad Central de Venezuela, Caracas, Venezuela. Haloperidol, 6-hydroxydopamine and desmethylimipramine were purchased from Sigma Chemical Company (St Louis, MO, USA).

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Experimental protocol. Adult male Sprague-Dawley rats, 230–290 g, were housed under controlled conditions of temperature and light (lights on from 0600 to 1800 h) and provided with free access to laboratory chow and water. A cannula (Severs et al 1970) was implanted in the left lateral cerebroventricle, 1 mm caudal to the coronal suture and 1·5 mm lateral to the midsagittal suture, with the aid of a stereotaxic instrument and under pentobarbitone anaesthesia (40 mg kg^{-1}, i.p.). The cannula was secured to the skull with acrylic cement. A minimum of 2 days was allowed for recovery. Single intracerebroventricular injections were made with a Hamilton syringe fitted with a stop to prevent needle penetration past the cannula tip.

One day after ventricular cannulation, the animals were randomly distributed into two groups: the first group received a subcutaneous dose of haloperidol (2·5 and 1·25 mg kg^{-1}, n = 17) or vehicle (0·3% tartaric acid, n = 17) at 18 and 2 h before JA116a, respectively. The second group was treated with desmethylimipramine (25 mg kg^{-1}, i.p.) 30 min before intracerebroventricular injection of 6-hydroxydopamine (250 μg/5 μL) or vehicle (0·9% NaCl (saline), 0·1% ascorbic acid, 5 μL), 72 and 48 h before JA116a injection (n = 20). 6-Hydroxydopamine treatment is a routine method for destroying catecholaminergic axon terminals in the central nervous system (Uretsky & Iversen 1969). This action is secondary to displacement of endogenous noradrenaline and appears to be due to intra-axonal oxidation of 6-hydroxydopamine, with consequent production of free radicals and mitochondrial damage (Uretsky & Iversen 1969; Kostrzewa & Jacobowitz 1974; Slivka & Cohen 1985). Entry of 6-hydroxydopamine into catecholaminergic axons relies on active transport by membrane processes that are normally responsible for endogenous amine re-uptake. Although we did not measure catecholamine content in the hypothalamus, selective central dopaminergic denervation and the noradrenaline protection in the rat was demonstrated using the same experimental protocol (Lenard et al 1991; Taghzouti et al 1991).

JA116a administration protocol. Animals were weighed and placed in metabolic cages. At 0900 h, half of the rats receiving each pretreatment were injected, as a bolus in 10 s, with saline (5 μL) or with freshly prepared JA116a in saline (100 μg/5 μL), followed by 20 mL kg^{-1} water orally. Urine was collected at 3 and 6 h; the bladder was emptied at 6 h by gentle suprapubic massage. Food and water were not available during the experiment. Ventricular cannula placement was confirmed postmortem by examining the distribution of an intracerebroventricular injection of 5 μL fast green dye, given before the animal was killed. Data were used only if the dye was distributed in the lateral, third, and fourth ventricles. Urine samples were assayed for sodium and potassium by flame photometry.

All data are presented as mean ± s.e.m. Statistical differences

between groups were analysed using two-way analysis of variance and by the Newman-Keul's range statistics.

Results

The effect of JA116a on urine response is illustrated in Table 1. Two-way analysis of variance and Newman-Keul's test revealed that JA116a evoked diuresis at the 3- and 6-h periods of collection. This effect was prevented by haloperidol pretreatment. Interaction between pretreatment and JA116a injection was significant at 3 h ($F = 5{\cdot}03$; $P < 0{\cdot}05$). The increase in urinary volume was associated with an enhanced natriuresis at 3- and 6-h periods of collection. This effect was also prevented by haloperidol pretreatment. There was a significative interaction between treatment and JA116a injection at 3 h ($F = 4{\cdot}8$, $P < 0{\cdot}05$). JA116a-induced kaliuresis was significant only at the 3-h period of collection and was not altered by haloperidol treatment. Evaluation of the urinary sodium to potassium ratio revealed no significant effect of JA116a at 3 and 6 h.

Results on JA116a central action in hydrated rats treated with desmethylimipramine, 30 min before intracerebroventricular administration of 6-hydroxydopamine, are presented in Table 2. In this experimental group, JA116a significantly increased urine volume at 3 and 6 h; this effect was inhibited by combined 6-hydroxydopamine and desmethylimipramine treatment ($F = 4{\cdot}5$, $P < 0{\cdot}05$). Evaluation of sodium excretion revealed a significant increase at 3 and 6 h. Central dopaminergic denervation with 6-hydroxydopamine and desmethylimipramine pretreatment reduced sodium excretion and inhibited JA116a-induced natriuresis. There was a significant JA116a and pretreatment interaction term (F values: 3 h = 8·4, and 6 h = 8·9, all P values < 0·01). Absolute potassium excretion was lower in all pretreatment groups and JA116a-induced kaliuresis was significant at 3 and 6 h. Evaluation of the urinary sodium : potassium ratio revealed a significant effect of JA116a at 3 and 6 h (F values: 3 h = 23·5 and 6 h = 19·9, all P values < 0·0001). Dopaminergic denervation blocked JA116a-induced urinary sodium : potassium increase (F values: 3 h = 11·6 and 6 h = 26·8, all P values < 0·0001).

Discussion

The present study further demonstrates that central administration of the novel compound JA116a increases urine volume and sodium excretion, an effect that is initiated only 3 h after intracerebroventricular injection of the compound. The delayed effect of JA116a might indicate that for its action this compound requires to be transported from the ventricle to the target organ or even out of the ventricular system to the cisternae on the brain external surface, ultimately affecting numerous intracerebral sites. Renal action of JA116a would reflect the total contribution of various brain areas as arcuate nucleus, median eminence, paraventricular nucleus and pituitary gland among other structures anatomically proximate and known to have a high density of dopaminergic neurons or receptors (Fuxe 1963; Saavedra et al 1975; Björklund & Lindvall 1978; Dawson et al 1986; Bouthenet et al 1987). Alternatively, the time-delayed effect of JA116a might have required the generation of a second messenger, ultimately leading to physiological effects.

The specific mechanism underlying JA116a renal action is unknown. Several lines of evidence imply that JA116a may exert its effects by an action on the neural dopaminergic components of the hypothalamus-pituitary axis, where dopamine has been proposed to participate in the control of fluid balance (Wolny et al 1974; Vandeputte-Van Messom & Peeters 1979; Forsling & Williams 1984) and vasopressin secretion (Bridges et al 1976). It has been found that intraventricular injection of dopamine into rats with normal water balance increases urinary volume (Wolny et al 1974) and inhibits vasopressin release (Forsling & Williams 1984). In addition, it was reported that dopamine-induced increases in renal blood flow are associated with increases in sodium excretion (Goldberg & Weder 1980; Torres et al 1989). Thus, a significant role for dopamine in the co-ordinated central and peripheral control of fluid and electrolyte homeostasis may be proposed.

The involvement of the brain dopaminergic system in the centrally mediated renal action of JA116a is strongly supported by our present results. The inhibitory effect of haloperidol on the natriuretic and diuretic response to intracerebroventricular administration of both JA116a and dopamine (Torres et al 1989;

Table 1. Urinary response to intracerebroventricular administration of JA116a in rats pretreated with haloperidol.

	Urinary volume (mL/100 g)		Sodium excretion (μEq/100 g)		Potassium excretion (μEq/100 g)		Na^+/K^+ ratio	
	3 h	6 h	3 h	6 h	3 h	6 h	3 h	6 h
Vehicle + saline	1·36 ± 0·16	2·54 ± 0·44	71·3 ± 16	176·3 ± 27	103·5 ± 20	239·1 ± 41	0·83 ± 0·1	0·79 ± 0·1
JA116a	2·69 ± 0·29*	3·82 ± 0·35*	183·7 ± 26*	332·9 ± 17*	200·5 ± 24•	314·8 ± 31	0·96 ± 0·07	1·08 ± 0·1
Haloperidol	1·45 ± 0·14	2·02 ± 0·16	108·9 ± 17	211·5 ± 34	122·4 ± 18	206·8 ± 25	0·82 ± 0·09	0·91 ± 0·07
Haloperidol + JA116a	1·75 ± 0·19	2·56 ± 0·27	121·8 ± 26	288·8 ± 49	188·9 ± 45	278·3 ± 49	1·31 ± 0·14	0·93 ± 0·15

*$P < 0{\cdot}05$ compared with all other groups, •$P < 0{\cdot}05$ compared with vehicle.

Table 2. Renal response to central administration of JA116a in rats with selective dopaminergic denervation with intracerebroventricular 6-hydroxydopamine treatment in combination with desmethylimipramine.

	Urinary volume (mL/100 g)		Sodium excretion (μEq/100 g)		Potassium excretion (μEq/100 g)		Na^+/K^+ ratio	
	3 h	6 h	3 h	6 h	3 h	6 h	3 h	6 h
Vehicle + saline	1·17 ± 0·27	1·97 ± 0·26	89·3 ± 16	177·3 ± 22	91·3 ± 19	182·1 ± 24	1·05 ± 0·06	0·97 ± 0·02
JA116a	2·53 ± 0·32*	3·75 ± 0·34*	172·5 ± 22*	264·5 ± 36*	142·1 ± 15*	262·4 ± 42**	1·21 ± 0·05***	1·12 ± 0·07***
Haloperidol	0·75 ± 0·29	1·35 ± 0·35	68·6 ± 17	133·2 ± 27	70·1 ± 18	131·6 ± 27	0·98 ± 0·09	1·01 ± 0·02
Haloperidol + JA116a	1·34 ± 0·29	1·86 ± 0·33	54·1 ± 8	88·8 ± 14	57·4 ± 7	95·0 ± 15	0·95 ± 0·08	0·94 ± 0·06

*$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$ compared with all other groups.

present results), and the failure of intracerebroventricular JA116a to exert its diuretic and natriuretic action when endogenous dopamine levels are reduced (Kostrzewa & Jacobowitz 1974; Slivka & Cohen 1985; Lenard et al 1991) points to a potentially important interaction between this compound and brain dopaminergic neurons. The possibility that JA116a regulates urine volume and sodium excretion when administered centrally due to an action on the hypothalamus to stimulate dopamine turnover must be considered. JA116a may stimulate tuberoinfundibular dopaminergic neurons to increase dopamine release from terminals in the median eminence. Dopamine then enters the portal capillaries and in the anterior pituitary exerts a tonic inhibitory action on prolactin release. It has been shown that prolactin contributes to the regulation of fluid and electrolyte metabolism in mammals and acts synergistically with angiotensin II to cause drinking and fluid retention (Horrbin 1980; Kaufman 1981; Loretz & Bern 1982). In this regard, turnover studies provide further evidence. An increase in dopamine-turnover has been observed during suckling (Fuxe et al 1969) or dehydration (Shoemaker & Schlumpf 1977) in the dopaminergic tuberoinfundibular neurons projecting to the posterior pituitary. Dopamine content of the neurohypophysis seems to correlate with hormone secretion, with an increase in lactating rats during suckling and in water-deprived rats (Holzbauer et al 1978). These results may suggest the existence of a dopaminergic hypothalamic control of the afferent pathway to neurosecretory cells which may be altered by JA116a. Alternatively, JA116a may stimulate a dopamine tuberohypophyseal system whose axons terminate in the neurohypophysis (Björklund & Lindvall 1978; Moore & Demarest 1982). In the pituitary posterior lobe, dopamine has been proposed to be involved in the water balance regulation (Forsling & Williams 1984).

In summary, our data suggest a contribution of the brain dopaminergic system to the expression of central JA116a-mediated renal effects. It seems possible that JA116a is able to modify the activity of the tuberoinfundibular or tuberohypophyseal dopamine neurons inducing in this way changes in fluid and electrolyte balance.

The authors wish to thank Dra Margarita Salazar-Bookaman for her helpful comments.

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