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Chronic treatment with mianserin prevents DOCA–salt hypertension in rats—evidence for the involvement of central 5-HT_2 receptors

Andréia L.S. Silva[a], Antonio M. Cabral[a], Gláucia R. Abreu[a], José G.P. Pires[a], Nazaré S. Bissoli[a], Andrew G. Ramage[b,*]

[a]*Departamento de Ciências Fisiológicas, Centro Biomédico, Universidade Federal do Espírito Santo, Av. Marechal Campos 1468, Vitória, ES 29040-090, Brazil*

[b]*Department of Pharmacology, University College London, Hampstead Campus, Rowland Hill Street, London NW3 2PF, UK*

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Abstract

Central 5-HT_{2A} receptors have been implicated in central volume control by activating a central angiotensinergic pathway to cause the release of vasopressin. Interestingly, to induce DOCA–salt hypertension in rats vasopressin release is required. Thus the present experiments were carried out to determine whether continuous blockade of these receptors over 20 days, with the non-selective 5-HT_2 receptor antagonist mianserin would prevent the development of deoxycorticosterone acetate (DOCA)–salt hypertension. Mianserin, given i.c.v. 90 or 60 μg twice daily for 20 days prevented the development of hypertension in conscious rats receiving DOCA–salt but did not affect blood pressure in rats on salt alone. Further, the dose of 30 μg given i.c.v. twice daily had no effect nor did the vehicle, polyethylene glycol (PEG), on the development of the hypertension. Mianserin 90 μg twice daily i.c.v. was also shown to prevent the increase in fluid intake, urinary flow and sodium excretion caused by DOCA–salt treatment. These data indicate that this action of mianserin is not due to an intrinsic hypotensive action but an action which involves interference with the mechanism by which DOCA–salt treatment causes hypertension. Thus the data overall support the view that to induce hypertension with DOCA–salt a central 5-HT-containing pathway needs to be activated, which then activates 5-HT_2 receptors to cause the release of vasopressin which has previously been shown to be responsible for the initiation of DOCA–salt treatment hypertension.

Keywords: 5-hydroxytryptamine; Deoxycorticosterone acetate; Experimental hypertension; 5-HT_2 receptor; Mianserin

1. Introduction

In conscious and anaesthetized rats activation of central 5-HT_{2A} receptors causes an initial rise in blood pressure and renal sympathoinhibition due to vasopressin release (Anderson et al., 1992, 1996; Pergola et al., 1993; Knowles and Ramage, 1999). Further, this 5-HT_{2A} receptor mediated release of vasopressin is caused by activation of a central angiotensinergic pathway (Saydoff et al., 1991; Knowles and Ramage, 1998). This has led to the suggestion that a central 5-HT containing pathway via activation of 5-HT_2 receptors may play a role in the control of blood volume (see Ramage, 2001). Interestingly, it has been known for some time that vasopressin is essential for the development of deoxycorticosterone acetate (DOCA)–salt hypertension in rats (Crofton et al., 1979; Berecek et al., 1980; Saito and Yajima, 1982; Liang et al., 1997). Hence the present study was carried out to determine if blockade of central 5-HT_2 receptors would prevent the development of DOCA–salt hypertension in rats. Mianserin was chosen to block these receptors as it is a non-selective 5-HT_2 receptor antagonist having pKi at 5-HT_{2A} receptors of 8.4, 5-HT_{2B} of 8.3 and 5-HT_{2C} of 8.3 (see Bonhaus et al., 1995) and it is freely available in large enough amounts to do long term studies. Mianserin was administered intracerebroventricularly (i.c.v.) twice daily over 20 days in uninephrectomized rats treated

* Corresponding author. Tel.: +44 20 7830 2898; fax: +44 20 7830 2327.
E-mail address: a.ramage@ucl.ac.uk (A.G. Ramage).

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with DOCA or vehicle and the effects of this treatment on their blood pressure and heart rate were compared after 20 days. In addition, daily measurements of fluid intake, urinary flow and sodium excretion were made.

2. Materials and methods

2.1. Animals

All animal procedures followed were in accordance with Biomedical Research Guidelines for the Care and Use of Laboratory Animals, as stated by the Federation of Brazilian Societies of Experimental Biology (FeSBE). 56 Male Wistar rats, initially weighing 150–200 g, from our breeding stock, were used. The animals were housed in a temperature-and humidity-controlled room (25 °C) with a 12-h light/dark cycle. During the experimental period, the animals were individually housed in metabolic cages for 24-h urine collection. Standard rat chow (Na^+ content 163 mEq/kg) and tap water were available ad libitum.

2.2. Surgery: implantation of cannula and kidney removal

For i.c.v. injections a stainless steel cannula guide was implanted into the right lateral cerebral ventricle under recovery anaesthesia with chloral hydrate (400 mg/kg, i.p.). The cannula was positioned 0.3 mm posterior to the bregma, 1.3 mm lateral to the midline and 4.5 mm below the skull surface, and then fixed with dental cement. The correct localization of the cannula was checked by observation of the movement of the saline solution meniscus in the pipette connected to the cannula through a PE 50 tube. The volume used for the i.c.v. injections was 3 μl. After implantation of this guide cannula all animals, still anesthetized, underwent uninephrectomy (flank incision, right side). All animals then received penicillin G benzathine (80,000 units, i.m.) and were allowed to recover.

For the measurement of mean arterial blood pressure and heart rate in these rats under freely moving conditions, a catheter was placed into the femoral artery filled with heparinized (40 IU/ml) saline and tunnelled to exit at the back of the neck under recovery anaesthesia with ether. These measurements were carried out at the end of the drug treatment period (i.e., the 21st day, excluding the initial surgical recovery period). The cannula was connected to a pressure transducer (P23XL, Statham, Valley View, OH, USA) coupled to a Biopac System (MP100, Santa Barbara, CA, USA) for the measurement of these variables 6 h after surgery.

2.3. Pretreatments and experimental protocols

Five days after the surgery, treatment regimes were initiated, DOCA (15 mg/kg, s.c.) or vehicle (soybean oil; 0.25 ml per animal, s.c.) was administered twice a week for 20 days beginning on Day 1. All rats were provided with drinking water containing 1% NaCl and 0.03% KCl from Day 0. Some rats also received twice daily, mianserin i.c.v. (30, 60 or 90 μg per rat) or 3 μl of mianserin vehicle (10% polyethylene glycol (PEG) in saline.) beginning concomitantly with DOCA or DOCA-vehicle. Rats were divided into 8 groups of which 5 were controls. The 5 control groups were DOCA vehicle–salt, DOCA vehicle–salt plus i.c.v. PEG, DOCA–salt plus i.c.v. PEG, DOCA vehicle–salt plus i.c.v. mianserin 90 mg, DOCA–salt. The 3 mianserin groups were DOCA–salt plus i.c.v. mianserin 30, 60, and 90 μg. In all cases, the i.c.v. injections were made twice a day, for 20 days. One day before (DAY 0) and during the experimental period, daily water intake and urinary sodium content and volume were determined as previously described (Bissoli et al., 2000).

2.4. Data analysis

Urine volume was determined gravimetrically and sodium concentration was measured by flame photometry (Micronal B262, São Paulo, Brazil). All data are expressed as means ± S.E.M. Unless otherwise stated, the data were statistically analysed using repeated measures analysis of variance (ANOVA) for the main effects, and Fisher test for paired comparisons among the means. Statistical significance was set at $P<0.05$.

2.5. Drugs

The drugs used were mianserin HCl, deoxycorticosterone acetate (DOCA), chloral hydrate and ether from Sigma Chemical Co., St. Louis, MO, U.S.A. and polyethylene glycol (PEG) and soybean oil from Sadia (São Paulo, Brazil). Mianserin was dissolved in 10% PEG in saline.

3. Results

All rats received salt in their drinking water (1% NaCl and 0.03% KCl) for 21 days.

3.1. Effect of deoxycorticosterone acetate (DOCA) and vehicles (soybean oil s.c. for DOCA and PEG i.c.v for mianserin; Table 1) on blood pressure and heart rate

The mean blood pressure after 20 days in rats receiving DOCA–salt (15 mg/kg, s.c.; $n=5$) was 150 ± 5 mmHg and this was associated with a heart rate of 358 ± 17 beats/min. However, in rats (control) receiving vehicle alone (soybean oil; s.c.; $n=5$) and salt their blood pressure was significantly ($P<0.001$) lower, see Table 1. In rats receiving PEG (10%, i.c.v. twice daily) and DOCA–salt ($n=8$) for 20 days their blood pressure was similar to that of DOCA–salt alone (see Table 1) but was significantly greater than DOCA–salt rats receiving twice daily 10% PEG (3 μl; i.c.v.; $n=5$).

Table 1
Effects of chronic i.c.v. injection (twice a day for 20 days) of mianserin (30, 60 or 90 μg in 3μl) or 10% PEG (3 μl) on mean arterial pressure (MAP) and heart rate (HR) in control and DOCA–salt hypertensive rats

Experimental groups	*n*	MAP (mmHg)	HR (beats/min)
DOCA vehicle–salt (control)	5	116 ± 5^{bb}	376 ± 7
DOCA vehicle salt + PEG (control)	5	114 ± 5^{bb}	357 ± 18
DOCA vehicle salt + mianserin 90 μg (control)	9	121 ± 9^{bb}	381 ± 13
DOCA–salt	5	150 ± 5^{aa}	358 ± 17
DOCA–salt + PEG	8	152 ± 6^{aa}	360 ± 20
DOCA–salt + mianserin 30 μg	7	151 ± 7^{aa}	374 ± 19
DOCA–salt + mianserin 60 μg	7	128 ± 6^{bb}	370 ± 30
DOCA–salt + mianserin 90 μg	10	126 ± 3^{bb}	357 ± 21

$^{a}P<0.05$; $^{aa}P<0.01$ when compared to DOCA vehicle–salt + PEG. $^{b}P<0.05$; $^{bb}P<0.01$ when compared to DOCA–salt + PEG group.

There were no significant differences between blood pressures in control rats receiving PEG (i.c.v.; $n=5$) and normal controls. The associated heart rates we not significantly different between any of these groups, see Table 1.

3.2. Effect of mianserin i.c.v. on blood pressure and heart rate

Neither, mianserin (90 μg twice per day; $n=9$) nor vehicle (PEG) given i.c.v. twice daily to control–salt rats over 20 days had significant effects on blood pressure, 121±9 and 114±5 mmHg, respectively compared with 116±4 mmHg for control–rats, (receiving no i.c.v. injections) and heart rate when compared to that of control rats. This dose regimen of mianserin given to DOCA–salt rats ($n=10$), however, prevented the development of hypertension and after 21 days the blood pressure was 128±3 mmHg and was not significantly different from vehicle controls (Table 1). Pretreatment with a lower dose of mianserin 60 μg twice per day had a similar effect (Table 1), while a dose of 30 μg twice per day did not affect the development of hypertension (Table 1). PEG i.c.v. (mianserin vehicle) failed to affect the development of hypertension in DOCA–salt rats (DSP). Again the associated heart rates were not significantly different between groups.

3.3. Effect of mianserin 90 μg twice per day i.c.v. on fluid intake, urinary flow and sodium excretion

The intake of fluid in control–salt rats was fairly constant from day 0 (67±5 ml) to day 20 (75±26 ml). In DOCA–salt rats fluid intake was also similar between day 0 (75±20) and day 20 (81±10 ml), however on beginning the DOCA injections there was a decrease in fluid intake which became significant on Day 2 (45±11 ml) but returned to Day 0 values by the next day, see Table 2. This decrease was also observed again on Day 8 and for both these periods was prevented by mianserin treatment. In DOCA–salt treated rats from Day 9 through to Day 13 there was a significant increased fluid intake compared with vehicle alone control–salt rats. This increase was again prevented by mianserin. Furthermore, in these DOCA–salt mianserin treated rats fluid intake was significantly reduced compared to the DOCA–salt rats on Days 9, 10, 13, 14 and from Day 16 onwards. Interestingly, in control–salt rats treated with mianserin (CSM90) there was tendency of fluid intake to decline over the experimental period, which was significant from Day 16 onwards. Overall, fluid intake in DOCA–salt rats receiving mianserin was similar to that of control–salt rats.

The urinary flow associated with the above fluid intake in control–salt rats was 20±2 ml per day. Over the treatment period this did not significantly change being 24±4 ml on Day 20. This was similar in control–salt rats treated with mianserin, although when comparing urinary flow between these two groups, control–salt and control–salt plus 90 μg mianserin, the urinary flow was significant reduced between days 3 and 6 and between days 15 and 19. In rats treated with DOCA–salt urinary flow increased, doubling by Day 2 to 30±11 ml compared with 14±4 ml on Day 0 (see Table 2), this then increased to 49±17 ml by Day 20. Mianserin (90 μg twice daily) treatment prevented this increase in urinary flow except on Day 5. In fact, urinary flow in mianserin (90 μg twice daily) treated DOCA rats was not significantly different to that in control–salt rats over the

Table 2
Daily values of fluid intake, urinary flow and sodium excretion obtained from DOCA vehicle–salt+PEG (CSP) and DOCA–salt+PEG (DSP) rats chronically treated with i.c.v. injection of PEG 10% daily, and DOCA vehicle+ mianserin (CSM90) and DOCA–salt+mianserin (DSM90) treated chronically with i.c.v. injection of mianserin (90 μg twice daily) over 20 days

Day	Fluid intake (ml)				Urinary flow (ml)				Sodium excretion (mEq/kg)			
	CSP	CSM90	DSP	DSM90	CSP	CSM90	DSP	DSM90	CSP	CSM90	DSP	DSM90
0	67±5	71±12	75±20	68±13	20±2	20±6	14±4	16±3	22±4	20±4	24±7	18±2
1	56±13	55±7	46±13	54±10	26±8	19±2	25±6	21±5	27±7	18 ± 2^{a}	$37\pm6^{aa,c}$	25 ± 5^{bb}
2	68±21	59±7	45 ± 11^{c}	47±10	19±2	17±3	$30\pm11^{aa,cc}$	16±4	17±2	19±2	$36\pm8^{aa,c}$	21 ± 8^{bb}
3	56±7	61±10	78±21	47 ± 11^{a}	31±7	15 ± 3^{a}	$24\pm6^{a,c}$	20±4	22±4	17±4	26±6	19±7
4	54±14	52±8	67±17	51±8	28±8	12 ± 3^{aa}	31 ± 10^{cc}	22±3	24±4	15 ± 2^{a}	28±6	23±4
5	65±16	59±5	50±6	46±10	36±13	12 ± 4^{aa}	20 ± 4^{aa}	31 ± 14^{cc}	22±5	15±4	28±7	14 ± 3^{bb}
6	62±8	46±9	50±5	52±6	25±4	12 ± 4^{aa}	25 ± 2^{c}	17±6	20±4	17±4	26±4	16 ± 3^{bb}
7	46±9	58±5	47±11	51±8	20±9	15±4	24 ± 8^{c}	19±4	23±3	19±4	$37\pm4^{aa,c}$	17 ± 7^{bb}
8	46±5	45±9	42 ± 10^{c}	50±8	21±3	16±3	22±4	17±3	21±4	18±5	30 ± 5^{aa}	17 ± 5^{bb}
9	51±8	45±9	94 ± 24^{aa}	54 ± 9^{bb}	19±6	12 ± 2^{a}	$29\pm6^{aa,cc}$	23±4	18±5	17±4	$35\pm7^{aa,c}$	18 ± 4^{bb}
10	38±8	56±17	88 ± 23^{aa}	56 ± 5^{b}	16±3	18±2	$41\pm13^{aa,cc}$	24 ± 3^{bb}	20±3	17±4	32 ± 4^{aa}	16 ± 4^{bb}
11	40±7	57±7	71 ± 15^{a}	52±13	14±4	17±3	$38\pm9^{aa,cc}$	21 ± 5^{bb}	19±2	15±4	30 ± 6^{aa}	17 ± 2^{bb}
12	48±7	62±4	77 ± 11^{a}	50±7	19±3	20±2	$35\pm9^{aa,cc}$	22 ± 3^{bb}	17±3	21±4	$32\pm4^{aa,c}$	16 ± 3^{bb}
13	49±12	54±9	100 ± 25^{aa}	50 ± 5^{bb}	17±6	22±5	$45\pm12^{aa,cc}$	20 ± 2^{bb}	18±6	21±3	$34\pm5^{aa,c}$	25 ± 3^{bb}
14	55±10	54±6	73±20	43 ± 3^{b}	25±4	19±2	$40\pm11^{aa,cc}$	17 ± 2^{bb}	24±5	17±3	$43\pm6^{aa,cc}$	23 ± 3^{bb}
15	56±9	53±4	75±8	49±10	22±4	14 ± 3^{a}	$35\pm12^{aa,cc}$	16 ± 5^{bb}	20±1	18±4	$39\pm4^{aa,cc}$	16 ± 4^{bb}
16	59±8	39±8c	82±8	38 ± 5^{bb}	22±5	14 ± 3^{aa}	34 ± 9^{cc}	14 ± 5^{bb}	19±2	18±5	$36\pm2^{aa,c}$	15 ± 3^{bb}
17	51±4	44±2c	88 ± 18^{a}	47 ± 10^{bb}	22±3	12 ± 2^{aa}	$32\pm10^{aa,cc}$	13 ± 5^{bb}	20±2	17±3	$38\pm5^{aa,cc}$	16 ± 3^{bb}
18	76±10	$41\pm1^{a,c}$	86±26	40 ± 1^{b}	25±7	15 ± 2^{aa}	$47\pm15^{aa,cc}$	19 ± 4^{bb}	20±2	17±4	$56\pm5^{aa,cc}$	24 ± 7^{bb}
19	68±15	50 ± 4^{c}	95±26	50 ± 4^{bb}	27±4	14 ± 2^{aa}	$48\pm15^{aa,cc}$	17 ± 5^{bb}	22±6	18±3	$42\pm3^{aa,cc}$	21 ± 8^{bb}
20	75±26	48 ± 7^{c}	81±10	47 ± 7^{b}	24±4	17±2	$49\pm17^{aa,cc}$	21 ± 5^{bb}	20±2	22±4	$44\pm6^{aa,cc}$	26 ± 4^{bb}

Values are mean±SEM for the $n=6$ in all groups. $^{a}P<0.05$, $^{aa}P<0.01$ when DOCA vehicle+mianserin (CSM90) and DOCA–salt+PEG (DSP) are compared with CSP ; $^{b}P<0.05$, $^{bb}P<0.01$ when DOCA–salt+mianserin (DSM90) compared with DOCA–salt+PEG (DSP); $^{c}P<0.05$, $^{cc}P<0.01$ in group comparison with DAY 0.

experimental period and was significantly less than that observed in DOCA–salt rats from Day 11 onwards. Overall, mianserin treatment prevented this increased urinary flow caused by DOCA–salt treatment.

The sodium excretion in control–salt rats remained constant over the whole experimental period starting on Day 0 at 22±4 mEq/kg. This was similar to that observed in control rats receiving mianserin (90 μg twice daily), although when compared to control–salt rats Day 1 and 4 sodium excretion was significantly smaller. DOCA–salt treatment caused an increase in sodium excretion which was significant on Day 1 (37±1 mEq/kg) and which remained fairly constant around this level over the 20 days. This increase in sodium excretion was also significantly greater than that observed in control–salt rats at Days 1 and 2 and from Day 7 onwards. Mianserin treatment (90 μg twice daily) of these DOCA–salt rats prevented this increase in sodium excretion and it remained fairly constant over the whole period (see Table 2).

Overall, the mianserin treatment normalized the changes in fluid intake, urinary flow and sodium excretion caused by DOCA–salt treatment.

3.4. Effect of mianserin treatment on body weight gain over 20 days

All rats started off with a weight of between 179 and 186 g. Those that received vehicle instead of DOCA increased in weight from 196±2 to 257±12 g for those on salt alone, from 189±6 to 248±6 g for those salt alone and receiving i.c.v. PEG and from 186±11 to 239±9 g for those salt alone and receiving i.c.v. mianserin 90 μg twice daily. However, for all animals that received DOCA their weight remained the same even if they also received mianserin and was significantly ($P<0.01$) less that those rats receiving just salt after 20 days.

4. Discussion

The present data demonstrate that mianserin, a non-selective 5-HT_2 receptor antagonist, given i.c.v. over 20 days prevents the development DOCA–salt induced hypertension in rats. Further, mianserin only affected blood pressure in rats developing hypertension and not in those that received soybean oil instead of DOCA. This would indicate that this action is not due to an intrinsic hypotensive action of mianserin but by an action that involves the interference with the mechanism by which DOCA causes the development of hypertension. As indicated in the introduction, this model of hypertension requires the production of vasopressin so these data would be consistent with the hypothesis that central 5-HT containing pathways play a role in the control of vasopressin release through activation of an angiotensinergic pathway via AT_1 receptors. Indeed, it has been reported that only central blockade of angiotensin AT_1 receptors (Kubo et al., 2000; Park and Leenen, 2001) causes a fall in blood pressure, whereas peripheral blockade has no effect (Hilditch et al., 1994; Lacour et al., 1994) in DOCA–salt hypertensive rats.

The ability of mianserin to block the development of DOCA–salt hypertension was not dose related. This may suggest it is not a receptor mediated action. However, this may just reflect the gradual accumulation of mianserin at the lower dose which could cause the same level of block as the high dose and this dose could also be supra-maximal, as it was given chronically twice a day for 20 days. Although blood pressure was only measured after 20 days the medium dose (60 μg) could have had a slight delay on the prevention of the DOCA–salt hypertension. It would be obviously very interesting to know the brain and plasma levels of mianserin during this time period, however these were not measured.

As mianserin was administered over a long period it cannot be assumed that the drug only remained in the brain. It is highly likely that it was distributed throughout the body and thus from the study it cannot be concluded that mianserin has only a central action, it could be acting peripherally or having its action by acting at both sites. In this respect under the conditions of DOCA–salt hypertension, intravenous 5-HT causes vasoconstriction by mainly activating 5-HT_{2B} not 5-HT_{2A} receptors (Watts et al., 1996). In addition, the 5-HT_{2B} receptor antagonist LY-272015 given intravenously causes a fall in blood pressure in this model, whereas in normotensive rats no effect is observed (Watts and Fink, 1999) suggesting that tonic activation of peripheral 5-HT_{2B} receptors is responsible in part for the maintenance of the increased peripheral resistance in this hypertensive model. As this 5-HT_{2B} receptor antagonist was given i.v. this would favour a peripheral site of action by which mianserin may be preventing the development of DOCA–salt hypertension. However, the fact that it took at least 10 min before a significant fall in blood pressure was observed in these DOCA–salt hypertensive rats could be interpreted that LY-272015 needs to enter the CNS before it can have its action. Moreover, this hypotensive action of LY-272015 was not observed until 21 days after DOCA–salt hypertension had been initiated and was only observed at the dose of 3 mg/kg, whereas after 28 days, interestingly, it could be observed for the 1 mg/kg dose as well. Furthermore, as the increased peripheral resistance in DOCA–salt hypertension involves the tonic release of endothelin, vasopressin and activation of the sympathetic nervous system (Yu et al., 2001) it is difficult to envisage how this antagonist is interfering with these effectors by peripheral site of action. Presumably, LY-272015 is inhibiting the increase in sympathetic tone and/or maintained vasopressin release which would favour a central site of action. Indeed, in normotensive rats activation of central 5-HT_{2B} receptors causes renal sympathoexcitation, although blockade of this receptor does not cause a fall in blood pressure in these rats (Knowles and Ramage, 2000). However, in chronic studies it would be very difficult to ensure that the drug remained in or outside the CNS. Thus it is not possible to completely rule out a

peripheral site of action for mianserin although the circumstantial evidence points strongly towards a central site of action.

Mianserin also prevents the increase in fluid intake caused by DOCA injections. Further, in animals not receiving DOCA injections mianserin treatment caused a delayed decrease in fluid intake. However, blockade of 5-HT_2 receptors at the level of lateral parabrachial nucleus has been reported to increase water and salt intake evoked by i.c.v. angiotensin II (Menani et al., 1996). This contradiction may be explained by the fact that in the present experiment the production of angiotensin centrally may be blocked by mianserin, as activation of central 5-HT_2 receptors to produce vasopressin is blocked by central AT_1 receptor blockade (Knowles and Ramage, 1998). Thus this parabrachial system is not operating. Further mianserin also prevented the increase in urine flow and sodium excretion caused by DOCA injection. This in fact could be indirect as this increase could be related to the increase in blood pressure caused by DOCA and the prevention of this rise in blood pressure by mianserin pretreatment would prevent pressure-evoked diuresis. Interestingly, mianserin in control rats tended to decrease urinary flow with only slight effects on sodium excretion. However, how this relates to the suggested block by mianserin of vasopressin production is unclear as this would be expected to increase urinary flow. Thus it is difficult to relate the effects of mianserin on fluid intake and excretion especially, as some of these changes would be related to changes in blood pressure.

Thus the present experiments provide support for the view that a central 5-HT containing pathway utilising 5-HT_2 receptors is involved in blood volume control by activating a central angiotensinergic pathway which causes the release of vasopressin and other central cardiovascular mediated effects. Further, over activity in this system, especially related to 5-HT_2 receptors, could be related to development of certain types of hypertension. Nevertheless, a slight cautionary note should be added, in that mianserin is known to bind to both α_1- and α_2-adrenoceptors, histamine H_1 receptors and 5-HT_{1D} receptors (see Marek et al., 2003) and thus interference with these receptors cannot be ruled out in explaining the ability of mianserin to prevent the development of DOCA–salt hypertension, although the failure of mianserin to lower blood pressure in controls suggests that α-adrenoceptor blockade is not very effective. Thus more experiments using more selective 5-HT_2 receptor antagonists are now required to totally rule out the involvement of these receptors as well determining which subtype of 5-HT_2 receptor is involved.

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