Nacartocin — Analogue of Oxytocin with Enhanced Natriuretic Properties: Natriuretic and Hemodynamic Characteristics

P. HRBAS, J. ŠKOPKOVÁ, J. ZICHA*, T. BARTH, M. LEBL, K. JOŠT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Praha, Czechoslovakia

** Institute of Physiology, Czechoslovak Academy of Sciences, 142 20 Praha, Czechoslovakia*

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Nacartocin, [2-p-ethylphenylalanine]deamino-6-carba-oxytocin, is almost three times more potent than oxytocin as a natriuretic agent. The natriuretic effect is mainly due to the inhibitory action of the peptide on tubular sodium resorption. Nacartocin decreased the blood pressure of anesthetized rats by the decrease of the total peripheral resistance which was greater than that observed after oxytocin administration. In conscious rats, Nacartocin caused a slight but prolonged increase of blood pressure.

The mechanism of the natriuretic action of oxytocin and its analogues still remains to be clarified by physiological studies. Nevertheless, the search continues for analogues of neurohypophysial hormones with selectively increased natriuretic potency. After dealing with problems concerning the experimental approach and comparing the natriuretic effects of a number of analogues synthetized earlier [Hrbas et al. 1980a, b; Škopková et al. 1980], we prepared a series of analogues of deamino-6-carba-oxytocin modified in position 2. Some of these analogues were found to have increased specific natriuretic action [Barth et al. 1981; Lebl et al. 1982]. Of the two most active analogues, [2-p--methylphenylalanine]- and [2-p-ethylphenylalanine]deamino-6-carba-oxytocin, we chose the latter (Nacartocin — I. N. N. — WHO) for further studies because its natriuretic action was more specific.

This report presents a detailed study on the dependence of the excretion of sodium, potassium and creatinine on the dose of Nacartocin in conscious rats and describes the hemodynamic action of this compound in anaesthetized and conscious rats.

Nacartocin was prepared according to Lebl et al. [1980].

Assay of the natriuretic effect of Nacartocin: The assay was performed in conscious adult Wistar male rats obtained from Institute of Physiology, Czechoslovak Academy of Sciences, Praha [Cort et al. 1975, Lebl et al. 1982]. Five rats were kept per cage under 12 h light: dark cycle and fed with regular pelleted diet and tap water ad libitum. The experiments were preceded by an adaptation period during which the rats were habituated to the experimental procedure; a soft catheter was introduced into their stomach (without applying water), they were pricked subcutaneously with a needle in the inguinal region and kept for two hours in individual metabolic cages constructed so as to enable reliable separation of urine and faeces. This training procedure was repeated every other day for a week. On the day preceding the experiment, approximately at 16.00 h the food was removed, while water remained available. At the beginning of the experiment, the rats were weighed (the average weight was 200—210 g) and given tepid tap water by means of a stomach catheter; the water load was 4% of body weight. Standard hydratation ensured pronounced water diuresis with a maximum decrease of urine osmolarity, i.e. without the intervention of endogenous vasopressin, and made any antidiuretic effect of the tested compound easily discernible. Immediately after the water load, Nacartocin or oxytocin was applied subcutaneously in a constant volume of 1 ml/kg body weight in dose of 0.2, 1, 5 or 25 μg/kg body weight. The rats were then placed in individual metabolic cages and urine was collected in 30 min intervals for 5 h. The concentration of Na and K in the samples was determined using a flame photometer (ZEISS, Jena). The concentration of endogenous creatinine was determined according to Yatzidis [1972]. The results were subjected to regression analysis and t-test.

Determination of hemodynamic parameters: The effect of the peptides on the total hemodynamics was determined in conscious or anaesthetized male rats of the Wistar-Konárovice strain (250—280 g) using a technique described in detail by Zicha et al. [1982]. The catheters were implanted into the carotic artery and jugular vein of anaesthetized rats immediately before the experiment. The body temperature of the anaesthetized rats was maintained at 37.5 ± 1.5 °C by means of pulses of infrared rays under feed-back regulation by a rectal temperature. The conscious and relatively unrestrained animals were measured in plastic cages. The mean arterial pressure (MAP) was measured by mean of a MP 15 transducer (MICRON Instruments, U.S.A.) connected to a two-channel amplifier and recorder (HEWLETT-PACKARD, 321). The heart rate was taken from a fast recording (20 mm/s) of the pulse wave. The cardiac output was measured by the dye dilution technique [Albrecht et al. 1975]. The indicator (Cardiogreen, HWD, U.S.A.) was injected into the right atrium. Samples of arterial blood were drawn from the aorta at a rate of 1.1 ml/min and passed through the cuvette of measuring densitometer. After recording the dye dilution curve, the withdrawn blood was infused into the animal. The total peripheral resistance (TPR = MAP/CO, i.e. mm Hg per ml/min 100 g), stroke volume (SV = CO/HR) and central blood volume (CBV = CO . MTT) was calculated after each measurement of cardiac output. The mean transit time of the indicator (MTT) was the time that elapsed from the injection of indicator until the moment when one half of the area under the corrected dilution curve was just reached.

Table 1

Excretion of water, sodium, potassium and creatinine for 5 h after subcutaneous application of oxytocin Nacartocin and saline

Substance	Dose ($\mu g\ kg^{-1}$)	V ($ml\ kg^{-1}$)	$U_{Na}V$ ($meq\ kg^{-1}$)	U_KV ($meq\ kg^{-1}$)	$U_{Cr}V$ ($mg\ kg^{-1}$)	n
Oxytocin	0.2	45.8 ± 2.7	0.48 ± 0.11	0.48 ± 0.05	non-determined	5
	1.0	37.6 ± 2.0	0.78 ± 0.13	0.39 ± 0.05	3.49 ± 0.18	10
	5.0	43.8 ± 1.9	1.43 ± 0.13	0.48 ± 0.10	5.12 ± 0.30	11
	25.0	48.9 ± 6.5	2.60 ± 0.80	0.59 ± 0.17	6.51 ± 1.48	7
		$y = 0.32x + 3.56$	$y = 102.71x - 114.53$	$y = 7.29x + 30.29$	$y = 214.81x - 74.88$	
		$R^2 = 0.05$	$R^2 = 0.36$	$R^2 = 0.03$	$R^2 = 0.20$	
Nacartocin	0.2	45.8 ± 1.6	1.10 ± 0.13^b	0.70 ± 0.05^b	4.18 ± 0.17	10
	1.0	50.1 ± 1.3^a	2.48 ± 0.10^a	0.80 ± 0.04^a	4.69 ± 0.27	41
	5.0	50.8 ± 1.4^a	3.71 ± 0.17^a	1.04 ± 0.07^a	6.13 ± 0.25	47
	25.0	46.7 ± 1.9	4.22 ± 0.14^a	1.14 ± 0.06^a	6.79 ± 0.44	48
		$y = 0.10x + 5.16$	$y = 137.36x - 26.89$	$y = 23.33x + 37.15$	$y = 141.42x + 207.93$	
		$R^2 = 0.01$	$R^2 = 0.46$	$R^2 = 0.14$	$R^2 = 0.17$	
Saline	1 $ml\ kg^{-1}$.	37.6 ± 1.1	0.32 ± 0.03	0.27 ± 0.03	3.56 ± 0.31	19

a, b — significantly different ($P < 0.005$, $P < 0.01$, resp.) from the corresponding doses of oxytocin.

Table 2

Changes of individual hemodynamic parameters (as percentage of control values) after the application of oxytocin and Nacartocin to anaesthetized rats

Substance	Time (min)	MAP	CO	TPR	HR	SV	CBV
Nacartocin	5	-5.8 ± 1.2^{b}	21.2 ± 9.1	-22.0 ± 5.3^{b}	1.5 ± 2.1	19.5 ± 11.4	10.3 ± 2.2^{ba}
0.5 µg kg^{-1}	10	-3.8 ± 2.1	31.1 ± 5.5^{bA}	-38.2 ± 2.7^{Ba}	0.6 ± 2.5	31.6 ± 6.4^{Ba}	23.3 ± 6.3^{ba}
(n = 4)	30	-8.8 ± 5.3	25.5 ± 21.3	-21.5 ± 13.2	4.7 ± 2.2	22.2 ± 22.5	6.3 ± 9.4
Nacartocin	5	-11.4 ± 2.7^{b}	8.5 ± 11.9	-18.7 ± 7.4	-6.3 ± 1.6^{b}	16.2 ± 11.6	7.4 ± 10.3
5 µg kg^{-1}	10	-12.5 ± 3.5^{b}	15.4 ± 5.9	-22.7 ± 5.1^{b}	-3.8 ± 1.1^{b}	16.3 ± 1.0^{Ba}	-4.7 ± 2.6
(n = 3)	30	-13.4 ± 5.2	58.5 ± 31.6	-39.6 ± 12.7^{b}	1.2 ± 5.5	49.2 ± 18.5^{a}	12.6 ± 16.0
Oxytocin	5	-3.6 ± 1.8	6.7 ± 10.6	-6.3 ± 11.4	4.3 ± 3.6	1.5 ± 8.2	-2.9 ± 3.6
0.5 µg kg^{-1}	10	-4.2 ± 2.5	1.3 ± 6.7	-4.5 ± 4.9	0.7 ± 3.4	0.6 ± 3.8	0.8 ± 6.1
(n = 4)	30	-11.8 ± 4.1	6.1 ± 13.3	-11.6 ± 11.3	2.8 ± 6.5	2.2 ± 8.7	4.2 ± 3.7
Oxytocin	5	-5.3 ± 2.3	1.9 ± 5.4	-4.8 ± 4.4	1.9 ± 4.7	3.1 ± 4.2	2.8 ± 4.1
5 µg kg^{-1}	10	-5.8 ± 2.1^{b}	4.2 ± 6.5	-8.5 ± 5.9	1.8 ± 5.3	3.4 ± 4.6	6.6 ± 5.5
(n = 4)	30	-13.6 ± 6.1	3.7 ± 6.7	16.5 ± 3.9^{B}	-3.4 ± 3.9	7.2 ± 3.4	13.9 ± 9.1

a, A — significantly different ($P < 0.05$, $P < 0.01$) from oxytocin;
b, B — significantly different ($P < 0.05$, $P < 0.01$) from the initial level (100%).

The influence of Nacartocin on the circulation was tested in two types of experiment. In animals anaesthetized by tiobarbiturate (Inactin, PROMONTA, Hamburg; 100 mg/kg^{-1} i.p.), a comparison was made of the response to Nacartocin and oxytocin in doses of 0.5 and 5 μg kg^{-1} as measured 5, 10 and 30 min after the i.v. administration of the peptide. In conscious rats, the changes of hemodynamics were followed in the course of the natriuretic response, i.e. 30 and 60 min after s.c. administration of 5 μg kg^{-1} of Nacartocin. Controls were given saline injections.

Table 3

Changes of individual hemodynamic parameters (in percentage of control values) after the application of Nacartocin and saline to conscious rats

	Nacartocin 5 μg kg^{-1} (n = 7)		Saline (n = 5)	
	30 min	60 min	30 min	60 min
MAP	6.9 ± 1.4^{AB}	9.6 ± 1.7^{AB}	−5.7 ± 3.4	−3.4 ± 1.4
CO	6.8 ± 10.9	1.4 ± 9.2	10.0 ± 3.4^{a}	−8.8 ± 10.3
TPR	5.7 ± 11.2	12.3 ± 10.1	−14.7 ± 4.5^{a}	14.8 ± 19.3
HR	4.0 ± 3.7	1.3 ± 2.6	−4.0 ± 4.4	−2.9 ± 8.2
SV	1.7 ± 7.9	0.7 ± 9.7	16.9 ± 7.8	−0.3 ± 16.3
CBV	5.6 ± 7.6	9.6 ± 11.4	24.7 ± 15.2	9.9 ± 15.0

a, A — significantly different ($P < 0.05$, $P < 0.01$) from the initial level (100%);
B — significantly different ($P < 0.01$) from saline.
MAP mean arterial pressure (mm Hg).
CO cardiac output.
TPR total peripheral resistance (mm Hg . ml^{-1} . min . 100 g).
HR heart rate (beats/min).
SV stroke volume (μl/100 g).
CBV central blood volume (ml/100 g).

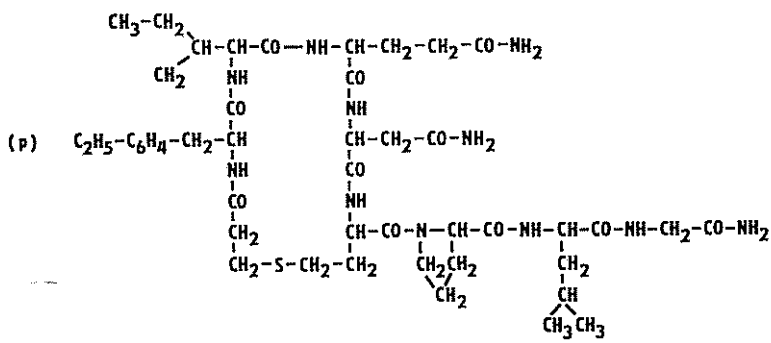

Fig. 1. Structure of Nacartocin — [2-p-ethylphenylalanine]deamino-6-carba-oxytocin.

Nacartocin caused almost three times higher sodium excretion and twice as high potassium excretion than equimolar oxytocin doses. The difference was significant for both ions at almost 1 % level (Tab. 1). Tab. 1 also presents regression equations of the dependence of diuresis and the excretion of sodium, potassium and creatinine on dose of oxytocin and Nacartocin.

After the application of Nacartocin in doses of 1 and 5 $\mu g\ kg^{-1}$, the diuresis was slightly higher than that after oxytocin, whereas $U_{Cr}V$, which we take to indicate the glomerular filtration rate (in spite of its only approximative value due to the fact of simultaneous tubular excretion of creatinine in rats), was practically unaltered. If the increase of $U_{Na}V$ and $U_{Cr}V$ after Nacartocin application was expressed as a percentage of the value obtained after the administration of a respective dose of oxytocin, then, with increasing doses of Nacartocin $U_{Na}V$ was higher by 129 %, 218 %, 159 % and 62 %, whereas $U_{Cr}V$ (omitting the lowest dose of oxytocin — see Tab. 1) was higher by only 23 %, 19 % and 4 %. This showed that sodium was presumably excreted mainly due to the inhibitory effect of Nacartocin on the resorption in the tubul and only to a lesser extent by its influence on glomerular filtration. The sodium increment in the tubular fluid brings about higher diuresis. The compound had a transient dose-dependent antidiuretic effect after which natriuresis began, reaching its maximum in the first stages of urine collection, and declining gradually afterwards.

In anaesthetized rats which were characterized by a low initial cardiac output and high peripheral resistance (MAP 121 $\pm$ 3.0 mm Hg, i.e. 16.1 $\pm$ 0.4 kPa; CO 25 + 1.3 ml min^{-1} per 100 g; TPR 5.1 $\pm$ 0.28 mm Hg or 0.68 $\pm$ 0.03 kPa . min . 100 g/ml; n = 15), Nacartocin had a depressor activity (Tab. 2). This effect, brought about by the decrease of the total systemic resistance, was more pronounced than the depressor effect of oxytocin. In a dose of 0.5 $\mu g\ kg^{-1}$, Nacartocin also transiently increased the cardiac output, stroke volume and central blood volume. The decrease of the mean arterial pressure was more significant after 5 $\mu g\ kg^{-1}$ than after 0.5 $\mu g\ kg^{-1}$, whereas the decrease of systemic resistance was not (Tab. 2).

In conscious rats (Tab. 3) which have high basal cardiac output and low systemic resistance (MAP 114 $\pm$ 3.2 mm Hg or 15.2 $\pm$ 0.4 kPa, CO 45 $\pm$ 3.0 ml min^{-1} per 100 g, TPR 2.7 $\pm$ 0.18 mm Hg or 0.36 $\pm$ 0.024 kPa . min . 100 g ml^{-1}; n = 12), Nacartocin had a low pressor effect. No other hemodynamic parameters investigated were significantly influenced by the administration of Nacartocin.

Discussion

Higher specific natriuretic activity of Nacartocin than that of oxytocin was repeatedly observed [Hrbas et al. 1980a, b; Škopková et al. 1980; Barth et al. 1981; Lebl et al. 1982]. It was therefore subjected to further studies in order to clarify the mechanism of its action in the kidneys and its influence on hemodynamic parameters. The determination of sodium, potassium, creatinine and water excretion at certain intervals after the application of individual doses made it possible to conclude that increased sodium excretion was brought about by higher tubular sodium rejection. Two modifications of the oxytocin molecule, namely absence of primary amino group and the 6-carba substitution may be considered responsible for increasing sodium excretion two to three times [Machová et al. 1975; Lebl et al. 1982]. The higher specificity of this effect could be attributed to the modification in position 2 [Lebl et al. 1982].

In the rat kidney medullary membrane system, Nacartocin had a slightly higher ability than oxytocin for binding and activation of adenylate cyclase [Buttlen et al. 1983]. In a similar system prepared from human kidneys, Nacartocin had a lower capacity for binding and activating adenylate cyclase [Guillon et al. 1982]. Consequently, Nacartocin would have to be given in higher doses than oxytocin in order to produce the required natriuretic effect in man. The measurement of hemodynamic parameters showed that Nacartocin, in doses that would be used clinically, did not decrease cardiac output and was not toxic to the cardiovascular system. The fact that Nacartocin had a depressor effect in anaesthetized rats and a pressor effect in conscious rats is not so surprising if we consider that the total peripheral resistance in barbiturate-anaesthetized rats is very high as compared to conscious animals [Salgado et al. 1976].

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