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Central regulation of intraocular pressure and cardiovascular apparatus with clonidine in conscious rabbits

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ABSTRACT Effects of clonidine on intraocular pressure (IOP), arterial blood pressure (BP) and heart rate (HR) were evaluated in normotensive conscious rabbits. Ocular and arterial hypotension and sinus bradycardia induced by intracerebroventricular (*icv* third ventricle) clonidine (0.1-5 μg/rabbit) were reduced by *icv* yohimbine (10 μg/rabbit), naloxone (4 μg/rabbit), bicuculline (2.5 μg/rabbit), acetylsalicylic acid (100 μg/rabbit) or flunoxaprofen (100 μg/rabbit). Our results suggest that α_2-adrenerg opiateic, peptidergic, GABAergic and prostaglandin systems are involved in ocular and cardiovascular effects induced by clonidine and that central nervous system is involved in the regulation of IOP.

KEY WORDS clonidine; intraocular pressure; blood pressure; heart rate; intraventricular injections; yohimbine; naloxone; bicuculline; lysine acetylsalicylic acid; flunoxaprofen

Clonidine, an α_2-adrenergic-stimulating agent, has been used for arterial hypertension therapy[1]. Clonidine, besides its hypotensive and bradycardic effects, determines reduction of intraocular pressure (IOP) when administered systematically or topically into the conjunctival sac[1-4]. We know, besides, that *icv* administration of clonidine determines in anesthetized rabbits ocular hypotension, suggesting a central nervous regulation of IOP[5].

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The aims of this work were to evaluate the participation of opiate peptidergic, GABAergic and prostaglandin (PG) systems in the ocular and arterial hypotensive effects and in sinus bradycardia induced by clonidine administered intracerebroventricularly (*icv*: 3rd ventricle) and to confirm the central nervous regulation of IOP.

METHODS

Experiments were carried out with normotensive conscious ♂ rabbits (2.9-3.2 kg). IOP was measured by a Schioetz tonometer that was previously calibrated for the rabbit eye by direct manometer[6].

Arterial blood pressure (BP) and heart rate (HR) were registered in the tail by a Digital Pressure Meter LE 5000 LETICA-Tecniplast Gazzada[7]. These measurements were taken 30 min after every clonidine administration. Drugs were given by *icv* (3rd ventricle). Each group consisted of 5 rabbits. Clonidine hydrochloride (Boehringer Ingelheim); yohimbine hydrochloride (Sigma, USA); naloxone hydrochloride (Crinos, Italy); (+)-bicuculline methochloride (Sigma); lysine acetylsalicylic acid (Maggioni, Italy); flunoxaprofen (Ravizza, Italy). All doses were indicated as base. Statistical analyses of results were performed[8]. The significance of differences between test rabbits and their controls was calculated using ANOVA between samples.

RESULTS

Effects of clonidine In rabbits 30 min

Tab 1. Intraocular pressure (IOP), arterial blood pressure (BP) and heart rate (HR) in conscious rabbits treated with clonidine (C). n = 5 rabbits, $\bar{x} \pm SD$. *p>0.05, **p<0.05, ***p<0.01

Drug (icv) μg/rabbit	IOP (kPa) Before	IOP (kPa) After	BP (kPa) Before	BP (kPa) After	HR (bpm) Before	HR (bpm) After
C 0.1	2.8±0.17	2.4±0.22*	17.3±1.56	14.1±1.16**	283±28	190±32***
C 1	2.8±0.62	2.2±0.46**	17.9±1.73	10.3±1.34***	290±23	180±25***
C 5	2.7±0.06	1.3±0.46***	17.7±1.16	7.9±1.56***	287±21	153±23***
Y	2.8±0.02	2.9±0.22*	16.7±1.11	17.2±1.34*	303±30	315±23*
Y+C 0.1	2.7±0.20	2.7±0.22*	17.1±1.78	16.9±1.34*	293±23	278±19*
Y+C 1	2.8±1.11	2.8±1.34*	17.7±1.56	16.7±1.34*	288±21	268±26*
Y+C 5	2.7±0.22	2.8±0.22*	17.3±1.34	15.5±1.56**	292±24	265±24*
N	3.0±0.22	3.6±0.04***	17.2±1.16	16.4±1.16*	287±25	281±23*
N+C 0.1	2.8±0.22	2.6±0.22*	17.6±1.56	16.7±1.16*	299±27	262±22**
N+C 1	3.0±0.22	2.9±0.22*	17.1±1.16	15.5±0.89*	285±24	233±31**
M+C 5	2.8±0.22	2.3±0.22**	16.7±1.16	14.4±1.34**	293±28	237±22**
B	3.0±0.20	2.7±0.15*	17.0±1.34	17.3±1.56*	301±21	303±23*
B+C 0.1	2.9±0.15	2.9±0.15*	17.0±1.16	15.5±1.16*	293±23	265±19**
B+C 1	3.0±0.56	3.1±0.29*	18.0±1.78	16.1±1.56**	298±21	251±25**
B+C 5	2.9±0.22	2.4±0.24**	17.0±0.89	14.0±1.16**	297±24	243±24**
ASA	3.0±0.02	3.5±0.09***	17.5±0.89	14.9±0.89*	301±31	280±21*
ASA+C 0.1	2.9±0.22	3.0±0.56*	17.0±1.16	15.9±2.01*	289±27	233±20**
ASA+C 1	3.0±0.09	2.8±0.22*	16.9±1.16	13.0±1.56**	320±24	241±28***
ASA+C 5	2.9±0.18	2.3±0.13***	16.5±1.16	11.4±1.78***	371±28	212±23***
F	2.9±0.13	3.3±0.15***	16.3±1.34	15.9±1.56*	293±23	275±22*
F+C 0.1	2.7±0.15	2.6±0.40*	16.7±1.16	15.7±1.16*	298±28	247±23**
F+C 1	2.8±0.07	2.6±0.26**	17.4±1.34	14.1±1.16**	295±29	230±26**
F+C 5	2.7±0.15	2.2±0.24***	17.0±1.16	11.7±1.56***	315±32	208±24***

Experiments with yohimbine (Y, 10 μg/rabbit), naloxone (N, 4 μg/rabbit), bicuculline (B, 2.5 μg/rabbit) lysine acetylsalicylic acid (ASA, 100 μg/rabbit) and flunoxaprofen (F, 100 μg/rabbit) icv 5 min before clonidine.

before clonidine treatment (0.1, 1 and 5 μg/rabbit icv) produced a dose-dependent reduction in IOP (-13.2, -23.8, -53.4%) in BP (-18.5, -42.2, -54.9%) and in HR (-32.9, -35.2, -46.7%, respectively) (Tab 1).

Role of α_2-adrenergic system The clonidine effects were completely antagonized by a pretreatment (5 min before clonidine) with yohimbine (10 μg/rabbit icv; for this dose there were no changes in IOP, BP and HR)(Tab 1).

Role of opiate peptidergic mechanism Naloxne 4 μg/rabbit icv 5 min before clonidine, partially reduced the effects of clonidine 0.1, 1 and 5 μg/rabbit. The antagonism of naloxone on ocular and cardiovascular effects induced by clonidine (5 μg/rabbit) was less intense than yohimbine (Tab 1). Naloxone alone increased the IOP by 20.5%.

Role of GABAergic mechanism Bicuculline 2.5 μg/rabbit icv; 5 min before clonidine, (for this dose there were no changes in IOP, BP and HR) significantly reduced the effects induced by clonidine (Tab 1).

Role of PG mechanism Lysine acetylsalicylic acid 100 μg/rabbit icv and flunoxaprofen 100 μg/rabbit icv (Tab 1) reduced the ocular hypotension and the sinus bradycardia induced by clonidine. However, the reduction of bradycardia was less than the

reductions of ocular and arterial blood hypotension. Besides, lysine acetylsalicylic acid and flunoxaprofen alone increased the IOP by 16.9 and 14.4%, respectively.

DISCUSSION AND CONCLUSIONS

Our previous studies demonstrated that systemic administration of clonidine in conscious rabbits determined systemic and ocular hypotension and bradycardia[2,9]. These results were in accordance to other studies[5] made on anesthetized rabbits treated with intravenous and ventriculocisternal perfusion of clonidine. These effects were due to central nervous regulation of IOP[4,5] and probably to a change in systemic hemodynamics[5].

Our investigations with icv clonidine on conscious rabbits revealed that the cerebral structures adjacent to the 3rd ventricle were involved in the regulation of IOP and in the cardiovascular effects of clonidine.

Our studies, like our previous researches on conscious and anesthetized rats[10-12], demonstrated that α_2-adrenergic, opiate peptidergic, GABAergic and PG brain systems were also involved in ocular and cardiovascular effects induced by clonidine in normotensive conscious rabbits.

The conclusion that an α_2-adrenergic mechanism is involved is based on the findings that yohimbine[1], a competitive antagonist of α_2-adrenergic receptors, antagonized intraocular hypotension, systemic hypotension and sinus bradycardia induced by clonidine.

Besides, naloxone, bicuculline, lysine acetylsalicylic acid and flunoxaprofen reduced ocular and cardiovascular effects induced by clonidine.

The involvement of opiate peptidergic system in the ocular and cardiovascular effects of clonidine is based on the findings that naloxone, a competitive antagonist of opiate receptors which does not influence α_2-adrenergic receptors in the brain[1,13], reduced the ocular hypotension, bradycardia and systemic hypotension induced by clonidine.

Bicuculline[1], a competitive antagonist of $GABA_A$ receptors, reduced ocular and cardiovascular effects induced by clonidine. It reduced also clonidine hypotension in freely moving and anesthetized ♂ rats with normal arterial pressure and with spontaneous or DOCA-hypertension. Since bicuculline[1] has no α_2-adrenoceptor blocking properties the inhibitory effect can not be attributed to blockade of α_2-adrenoceptors.

Our experimental findings with lysine acetylsalicylic acid and flunoxaprofen, administered with a dose regimen expected to produce a selective inhibition of PG cyclooxygenase[1,14], demonstrate that the hypotensive ocular and cardiovascular effects of clonidine are also due to stimulation of PG biosynthesis in the CNS of normotensive rabbits. Therefore, the elevation of IOP after icv lysine acetylsalicylic acid and flunoxaprofen alone suggest a central prostaglandinic regulation of IOP. Besides, our investigations suggest the presence of ocular hypotensive PG in the CNS while other studies demonstrat that PG administered topically or sistemically increased IOP[15].

Our results suggest that in central regulation of IOP the cerebral structures adjacent to the 3rd ventricle are involved.

Other experiments are in progress to see which cerebral areas are involved in the intraocular effects of clonidine and to evaluate, at different times since administration, its cardiovascular and intraocular effects.

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可乐定对清醒兔眼压及心血管系统的中枢调节

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提要　在清醒兔身上研究了可乐定对眼压(IOP)、动脉压及心率的影响。脑室注射可乐定(0.1-5 μg)所引引起的眼压和动脉血压过低及窦性心动过缓可被脑室注射育亨宾10 μg、纳洛酮4 μg、荷包牡丹碱2.5 μg、阿司匹林100 μg或氟诺洛芬100 μg所拮抗。本文结果表明：α_2肾上腺素、阿片肽、GABA和前列腺素系统参与了可乐定所引起的眼压及心血管效应，从而说明中枢神经系统参与了眼压的调节。

关键词　可乐定；眼压；心率；脑室内注射；育亨宾；纳洛酮；荷包牡丹碱；赖氨酸乙酰水杨酸；氟诺洛芬