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CA2+ MODULATORS AS ANTIDOTES TO IMIPRAMINE AND NEUROTRANSMITTER TOXICITY

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Abstract. Flunarizine and nimodipine, Ca2+ modulators which exert antagonist effects against catecholamines and serotonin and have specific action on the brain, were used as antidotes to imipramine toxicity in the rat. Imipramine, a tricyclic antidepressant, inhibits synaptic reuptake of catecholamines and serotonin. Flunarizine administered concurrently with imipramine increased survival time significantly ($p < 0.04$). After a lethal dose of imipramine (85 mg/kg) 5 out of 5 animals treated with flunarizine (2.37 ± 1.21 mg/kg in divided doses) and 4 out of 5 animals treated with nimodipine (0.36 ± 0.11 mg/kg) survived. The acute toxicity of imipramine might be related, in part, to drug-induced alteration in turnover of excitatory neurotransmitters which will induce intracellular Ca2+ accumulation and damage to vital organs. These toxic effects of endogenously produced neuroamines may be antagonized by nimodipine or flunarizine.

Introduction. The antagonist and antidote properties of a calcium entry modulator, nitrendipine, on the functional and morphological cardiac toxicity of cocaine have been reported (1-3). Cocaine, an indirectly acting sympathomimetic amine, inhibits the reuptake of catecholamines at the synapse (4,5). Concurrently the increased synaptic concentration of excitatory neurotransmitter activates the receptor-controlled calcium channel and symptoms of cardiac toxicity such as tachyarrhythmias, coronary vasoconstriction and myocardial infarction may occur (1,6,7). Nitrendipine, a Ca2+ modulator of the dihydropyridine class, which has specific vasodilator action on the coronary vessels, will neutralize the toxic effects of elevated synaptic concentrations of endogenous catecholamines which follow cocaine administration (1,3).

In the present study the effects of other Ca2+ modulators were tested as antagonists and antidotes to imipramine. Imipramine, a tricyclic antidepressant, inhibits the reuptake of norepinephrine (8,9) and serotonin (10), excitatory neurotransmitters which control vital organ functions. After inconclusive trials with nitrendipine and verapamil, flunarizine and nimodipine were selected among the currently available Ca2+ channel modulators as potential antidotes to imipramine toxicity because of their antagonist effects against catecholamines as well as serotonin (11,12). In addition, flunarizine and nimodipine also exert a specific action on the brain (13,14), the main target of imipramine.

Methods. Twenty-nine male Sprague Dawley rats weighing 300 ± 40 g were fitted, under ether anesthesia, with a catheter in the caudal artery. The catheter was connected to a constant infusion micropump and a pressure transducer. Every 30 seconds a recorder and microcomputer displayed and printed out heart rate, systolic, diastolic and mean blood pressures. In a selected number of experiments, ECG was also recorded from three standard leads. The awakened animal was placed in a restraining grid.

In the first series of experiments, 10 animals were administered an intraperitoneal lethal dose of 100 mg/kg of imipramine. Immediately following intoxication, 5 control rats were administered intraarterially 10 µl/min of isotonic

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TABLE I: Antagonist Effects of flunarizine and nimodipine against imipramine and its use as an antidote in the rat

Number of rats	Amount of I.P. imipramine	Fluid vol. administered	Treatment	Survival time
5	100 mg/kg	10 µl/min	None	14.6' ± 5.5
5	100 mg/kg	10 µl/min	flunarizine 0.1 mg/kg/min	129.0' ± 89[a]
4	85 mg/kg	1.80 ± 0.30 ml	None	17.0' ± 6.0
4	85 mg/kg	4.92 ± 0.72 ml[b]	flunarizine 2.37 ± 1.21 mg/kg[b]	> 24 hrs[c]
5	85 mg/kg	4.23 ± 0.61 ml[b]	nimodipine 355 ± 109 µg/kg	1: 75' 4: > 24 hrs[c]

a $p < 0.04$
b In fractionated amounts during the period of treatment (187 ± 86 min)
c Animals conscious and active

saline while 5 test animals received 0.1 mg/kg/min of flunarizine solution (which approximates a volume infusion of 10 µl/min intraarterially).

In the second series of tests, eight animals were administered intraperitoneally 85 mg/kg of imipramine. After mean blood pressure fell to 50 mm Hg, which occurred within 14 ± 9 min, 4 animals were treated with successive intraarterial bolus of 0.3 ml saline while 4 others were given a similar bolus of saline concurrently with flunarizine 375 ug/kg.

In the third series of experiments, 5 rats were administered 85 mg/kg of imipramine intraperitoneally. After mean blood pressure fell to 50 mm Hg, the animals were given successive intraarterial bolus of 0.3 ml saline concurrently with nimodipine for a total dose of 355 ug/kg.

In the fourth series, six animals were administered 85 mg/kg imipramine intraperitoneally. When mean blood pressure fell below 50 mm Hg, three rats were treated with verapamil at 0.6 mg/kg intravenously by means of slow infusion. Three other animals were treated with nitrendipine administered in a loading dose of 7.4 µg/kg followed by a constant infusion of 1.22 µg/kg/min.

Verapamil, provided by Biosedra (France) was dissolved in a 2.5 mg/ml aqueous solution. Ten milligrams of flunarizine, provided in crystal form by Janssen Pharmaceutica was dissolved in 20 ml of a solution containing 0.5 ml absolute ethanol, 1.5 ml polyethylene glycol, and 18 ml of isotonic saline. Nitrendipine and nimodipine, provided by Bayer Laboratory, were dissolved in solutions containing polyethylene glycol. The amounts of Ca2+ modulator administered were selected on the basis of the experimental and clinical doses which have been reported to give optimal pharmacological and therapeutic activity (15). Imipramine was provided by Ciba Geigy Laboratory in crystal form and was dissolved in buffered saline. Data recorded by the computer were statisticaly analyzed with a program for calculation of t-test.

Results. (Tables I,II,III). In the first series survival time of the saline-treated animals was 14.6 ± 3.5 min while the rats treated with flunarizine at 0.1 mg/kg/min had a survival time of 129 ± 89 min ($p < 0.04$, Table I). In the

TABLE II: Acute effects of imipramine administered intraperitoneally (85 mg/kg) on mean blood pressure and heart rate of the rat (N=4). Animals were treated with bolus of saline to restore blood pressure (1.8 ± 0.3 ml). Survival time was 17.0 ± 6 min.

		(minutes after imipramine administration)					
TIME	CONTROL	3	6	9	12[a]	15[b]	18[b]
Mean pressure (mm Hg)	107 ±15	59[c] ±17	46[d] ±14	41[d] ±11	47[c] ±10	51[e] ±24	49[e] ±35
Heart rate (min)	421 ±45	345 ±76	317[c] ±110	254[c] ±36	270[e] ±49	269[e] ±47	272[e] ±64

a Measurements on 3 surviving animals
b Measurements on 2 surviving animals
c $p < 0.05$
d $p < 0.005$
e $p < 0.025$

second series survival time of the saline-treated animals was 17.0 ± 6 min (Table II). Total volume of saline administered was 1.8 ± 0.3 ml. As in the first series, these animals developed intractable hypotension bradycardia and convulsions. Imipramine administration was associated with electrocardiographic changes. These were limited to inversion of QRS complex. No serious arrhythmias were recorded. The QT interval, when corrected for heart rate, was not changed.

The rats that died of imipramine toxicity presented lung congestion, acute enlargement of right auricle and ventricle, myocardial infarction and ischemic kidney and liver.

The three animals treated with verapamil did not survive longer than the control; survival of the three rats treated with nitrendipine did not exceed 60 minutes. The 4 rats treated with flunarizine and saline after their blood pressure had reached 50 mm Hg, survived (Table III). They were administered a total dose of 2.37 ± 1.21 mg/kg flunarizine and 4.92 ± 0.72 ml of saline over a period of 187 ± 86 min following onset of hypotension. This treatment was performed until mean blood pressure and heart rate were restored to control values. Twenty four hours after the procedure, the animals were awake and active. Animals treated with imipramine displayed significant decreases in heart rate as well as blood pressure until fatal outcome. Administration of flunarizine progressively corrected these changes which were no longer significant 200 minutes after onset of intoxication. Four of the 5 animals treated with nimodipine at 355 ± 109 µg/kg also survived (Table 3).

Discussion. The toxic effects of imipramine on the cardiovascular system have been documented in the present study which corroborates reports of others (15). Simultaneous and sustained decreases in blood pressure and heart rate induced by imipramine are indicative of an impairment of the baroreceptor mechanism which regulates heart rate as a function of blood pressure. Hypotension may also be attributed to imipramine-induced decrease in myocardial function which has been reported by others (16). Electrocardiographic recordings indicate that the toxic dose of imipramine administered in these acute experiments did not induce major dysrhythmias. Such an observation appears to be at variance with the many reports of severe dysrhythmias occurring in the case of imipramine intoxication. However, the latter observations were performed following a prolonged period of imipramine intoxication lasting several hours or several

TABLE III: Effects of flunarizine or nimodipine intraarterially administered in successive bolus after imipramine intoxication (85 mg/kg, intraperitoneally) on heart rate and mean blood pressure of 4 rats. Bolus of saline (total volume 4.92 ± 0.72 ml for flunarizine and 4.23 ± 0.61 ml for nimodipine) were also administered. The 4 animals treated with flunarizine survived 24 hours; 4 out of 5 animals treated with nimodipine survived 24 hours.

			(minutes after imipramine administration)								
TIME	CONTROL (0 min)	F1[a] ←----→	30	60	90	120	150	180	210[d]	240[d]	270[e]
Mean pressure (mm Hg)	91 ±13		77 ±14	72 ±15	65[b] ±17	64[b] ±18	67[c] ±12	71[b] ±13	67 ±10	78 ±17	76 ±16
Heart rate (min)	447 ±20		362[b] ±84	364[b] ±40	341[c] ±42	353[b] ±81	359[c] ±61	392[b] ±41	366[d] ±49	407 ±64	415 ±77
		←Nim----→									
Mean Pressure (mm Hg)	95 ±16		57[b] ±11	54[b] ±20	62[b] ±11	54[b] ±20					
Heart rate (min)	374 ±33		443[c] ±46	421[b] ±41	461[c] ±15	410 ±55					

[a] Flunarizine or nimodipine is administered during the first 20 minutes whenever mean blood pressure reaches 50 mm Hg, which varies from animal to animal
[b] $p < 0.05$
[c] $0.025 < p < 0.005$
[d] measurements on 3 animals
[e] measurements on 2 animals

days. In the present studies the period of lethal intoxication did not exceed 20 minutes. It appeared that other causes besides impairment of cardiac conduction contributed to the death of the animal. Post-mortem examination showed macroscopic alterations of liver, kidney and lung which all contributed to the death of the animals.

The protective effect of flunarizine and of nimodipine against lethal imipramine intoxication might be accounted for by their antiserotoninergic and anticatecholaminergic properties. Synaptic accumulation of these excitatory neurotransmitters will result in cardiac, pulmonary, renal and cerebral dysfunction.

The first effect of these Ca^{2+} modulators is to improve cardiac performance by decreasing peripheral resistance (17) and blood viscosity (18). The antiserotoninergic properties of nimodipine and of flunarizine should offset the untoward effects produced by elevated serotonin and norepinephrine concentration on the pulmonary circulation. These neurotransmitters are normally removed by the lung (19) and this removal is inhibited by imipramine or cocaine (20). Small changes in the magnitude of serotonin removed by the lung,

which normally amounts to 95%, could produce important changes in the serotonin concentration of arterial blood; a 10% decline in removal to 85% could result in a three-fold increase in concentration in the left atrium and systemic arterial blood (21). As a result the systemic vascular response to serotonin will be increased or prolonged. In the lung increased concentration of serotonin will induce pulmonary vasoconstriction, arteriovenous shunting and hypoxia which will further decrease serotonin clearance.

Flunarizine and nimodipine could, therefore, antagonize serotonin-induced and self-propagated impairment of lung function. These two Ca2+ modulators which cross the blood-brain barrier have been reported to improve cerebral blood flow (13,14). They will also interact in the brain with serotonin and catecholamines. Serotonin plays an important role in the central regulation of blood pressure by reducing cardiac sympathetic tone (22). Others have suggested that the hypotensive effect of serotonin may be due to enhanced activity in the bulbospinal serotoninergic pathway (23). Nimodipine and flunarizine could offset the stimulation by serotonin of these central pathways which control blood pressure. Inhibition of synaptic reuptake of catecholamines produced by imipramine may further compound all of the disturbances caused by impairment of serotonin turnover.

The present observations imply that the acute toxicity of imipramine might be related, in part, to drug-induced alteration in metabolism and turnover of excitatory neurotransmitters norepinephrine and serotonin which will result in their elevated synaptic concentration. In turn such disturbances induce intracellular Ca2+ accumulation with its accompanying cellular damage to vital organs. Endogenously produced excitatory neurotransmitters might then act like cellular toxins and their effects may be antagonized by Ca2+ modulators like nimodipine or flunarizine. However, because of the feed-back control mechanisms linking all brain neurotransmitters several of them might also be involved in imipramine toxicity and corrected by nimodipine or flunarizine.

The antidote properties of nitrendipine on the toxicity of cocaine (1-3) which inhibits synaptic reuptake of catecholamines, is another example illustrating the role of endogenous factors in the toxicity of certain psychoactive drugs. Imipramine was, in 1957, the first tricyclic antidepressant medication to be used clinically and the first case of lethal intoxication with this drug was reported two years later (24). At present, there is no specific treatment for tricyclic intoxication which accounts for up to 37% of all poison-related admissions to intensive care units (25). In this respect, the experimental observations herein reported might be of clinical interest for the treatment of imipramine toxicity.

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