EFFECT OF METHYLXANTHINES (THEOPHYLLINE, THEOBROMINE, CAFFEINE) AND CATECHOLAMINES ON NICOTINAMIDE COENZYME CONTENT IN THE RAT MYOCARDIUM

S. B. Frantsuzova

UDC 612.173.1.015.11:577.152.1'133]-06:547.857.4

In experiments on rats, after intraperitoneal injection of theophylline the content of oxidized forms of pyridine nucleotides (NAD+NADP) was reduced by 19.4%, the level of the reduced forms ($NAD \cdot H_2 + NADP \cdot H_2$) showed a tendency to decrease, and the total content of nicotinamide coenzymes was significantly reduced. After administration of caffeine the content of NAD+NADP and the total pyridine nucleotides showed a tendency to decrease. Theobromine had no significant effect on these parameters. The action of catecholamines and methylxanthines was compared. Isoproternol (exciting β-adrenergic receptors) lowered the NAD+NADP content, but adrenalin (25 μg/kg) increased the content of both oxidized (by 24%) and reduced (48%) forms of pyridine nucleotides. An increase in the dose of adrenalin (1000 μg/kg) led to a decrease in the level of oxidized forms (by 22.2%) and of the total nicotinamide coenzymes (by 18%).

KEY WORDS: catecholamines; adrenomimetics; methylxanthines; nicotinamide coenzymes; rat myocardium.

The important role of nicotinamide coenzymes in electron and hydrogen transport during cell respiration is firmly established [7]. However, only a few papers on the effect of cardiotropic and vasoactive drugs on the absolute and relative content of oxidized and reduced forms of nicotinamide coenzymes in the myocardium are to be found in the literature [2, 3, 5, 6]. The study of this problem is of considerable interest in order to explain the molecular basis of the regulatory action of cardiotropic drugs on the function of the cardiovascular system.

The writer has shown [4] that methylxanthines (theophylline, theobromine, caffeine) increase the catecholamine content in the myocardium 1 h after intraperitoneal injection and that this effect is accompanied by corresponding changes in the content of adenine nucleotides. The absolute and relative content of the pyridine nucleotides plays an important role in the synthesis of the latter.

The action of methylxanthines on the content of nicotinamide coenzymes in the myocardium was studied. The effect of methylxanthines was compared with the action of exogenous adrenomimetics: adrenalin, noradrenalin, and isoproterenol.

EXPERIMENTAL METHOD

In experiments on 106 rats of both sexes weighing 180–240 g the test drugs theophylline, theobromine, and caffeine were injected intraperitoneally in equimolar doses equivalent to 50 mg/kg theophylline, which is 10% of LD_{50} of this drug for albino mice by intraperitoneal injection [1]. It has been shown that in this

Central Research Laboratory and Department of Pharmacology, Kiev Medical Institute. [Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Cherkes (deceased).] Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 4, pp. 68–71, April, 1975. Original article submitted May 6, 1974.

TABLE 1. Content of Nicotinamide Coenzymes (in μg/g wet weight of tissue) in Rat Myocardium 1 h after Intraperitoneal Injection of Methylxanthines (M ± m)

Expt. No.	Substance	NAD + NADP	NAD · H_2 + NADP · H_2	NAD + NADP + NAD · H_2 + NADP · H_2	(NAD + NADP) / (NAD · H_2 + NADP · H_2)
1	Control	378±28	205,7±17,6	580,4±46,3	1,8±0,1
2	Theophylline (n=12)	304,26±16,8	178,9±8,04	480,6±21,26	1,69±0,1
	P_{1-2}	<0,05	>0,05	<0,001	>0,05
3	Theobromine (n=9)	362,5±17,44	229,2±10,15	592,3±24,3	1,57±0,067
	P_{1-3}	>0,05	>0,05	>0,05	>0,05
4	Caffeine (n=9)	320,7±19,04	194,8±10,49	515,5±15,6	1,67±0,11
	P_{1-4}	>0,05	>0,05	>0,05	>0,05

TABLE 2. Content of Nicotinamide Coenzymes (in μg/g wet weight of tissue) in Rat Myocardium after Intraperitoneal Injection of Catecholamines (M ± m)

Expt. No.	Substance	Dose (μg/kg)	NAD + NADP	NAD · H_2 + NADP · H_2	NAD + NADP + NAD · H_2 + NADP · H_2	(NAD + NADP) / (NAD · H_2 + NADP · H_2)
1	Control	—	388,7±16,85	201,9±14,1	590,5±24,8	2,01±0,13
2	Noradrenalin (n=9)	25	417,3±15,1	187,3±11,8	604,7±16,1	2,3±0,2
	P_{1-2}		>0,05	>0,05	>0,05	>0,05
3	Noradrenalin (n=8)	250	337,4±14	196,3±16	533,7±23,4	1,79±0,14
	P_{1-3}		>0,05	>0,05	>0,05	>0,05
4	Isoproterenol (n=9)	100	333,7±24	193,4±14,8	527,1±22,45	1,8±0,26
	P_{1-4}		=0,05	>0,05	>0,05	>0,05
5	Adrenalin (n=9)	1000	302,2±22	182,1±14,4	484,3±26,3	1,7±0,2
	P_{1-5}		<0,002	>0,05	<0,01	>0,01
6	Adrenalin (n=9)	250	407,4±14,2	235,2±13,8	642,6±24,4	1,8±0,1
	P_{1-6}		>0,05	>0,05	>0,05	>0,05
7	Adrenalin (n=8)	25	482,8±23,32	299,7±10,5	782,5±12,9	1,6±0,1
	P_{1-7}		<0,002	<0,001	<0,001	=0,05

dose theophylline effectively influences certain aspects of myocardial metabolism [8, 13, 15]. The animals were decapitated 1 h after injection of the substances. Catecholamines were injected intraperitoneally in doses affecting various metabolic processes in the myocardium [9, 12]: adrenalin 25, 250, and 1000 μg/kg, noradrenalin 25 and 250 μg/kg, isoproterenol 100 μg/kg. The animals were killed 20-30 min after injection of the preparations. The content of oxidized (NAD + NADP) and reduced (NAD · H_2 + NADP · H_2) forms of nicotinamide coenzymes was determined fluorimetrically [11]. The concentration of nicotinamide coenzymes was expressed in μg/g wet weight of tissue. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS AND DISCUSSION

The investigations showed that the rat myocardium contains chiefly oxidized forms of pyridine nucleotides, which in the control animals accounted for 65% of the total content of nicotinamide coenzymes. A similar ratio between oxidized and reduced forms has been observed by other workers in the myocardium of intact rats and rabbits [10]. This predominance of oxidized over reduced forms persisted after administration of the methylxanthines and catecholamines. Although by the method used only the total oxidized or reduced forms of pyridine nucleotides could be determined, the fact that animal tissues and, in particular, the myocardium contain chiefly NAD and NAD · H_2, and only very small proportions of NADP and NADP · H_2, respectively, was taken into consideration.

As Table 1 shows, 1 h after injection of theophylline the content of oxidized forms of pyridine nucleotides was reduced by 19.4% ($P<0.05$), the content of reduced forms by 13% ($P>0.05$), and the total content of nicotinamide enzymes was significantly reduced by 17%; compared with the control group of rats, however, the ratio (NAD + NADP)/(NAD · H_2 + NADP · H_2) was unchanged. After administration of caffeine the total content of pyridine nucleotides showed a tendency to fall, mainly at the expense of the oxidized forms.

The content of reduced forms showed no substantial change and the ratio (NAD + NADP)/(NAD • H_2 + NADP • H_2) showed a tendency to decrease. Theobromine had no significant effect on the content of pyridine nucleotides.

The decrease in the content of oxidized forms of pyridine nucleotides under the influence of theophylline and caffeine could be attributed both to a decrease in the biosynthesis of these nucleotides and to an increase in activity of the enzymes concerned with NAD degradation (NADase).

On the grounds that catecholamines, stored under the influence of methylxanthines, participate in the intracellular regulation of metabolism through the adenylate cyclase–3',5'-cyclic AMP system and also that methylxanthines, which inhibit cyclic nucleotide phosphodiesterase, increase the content of 3',5'-AMP [14], it was decided to compare the action of methylxanthines and of exogenous catecholamines.

As Table 2 shows, catecholamines in different doses affected the level of the pyridine nucleotides variously. Excitation of β-adrenergic receptors by isoproterenol was followed by a tendency for the total content of nicotinamide coenzymes to fall, mainly at the expense of oxidized forms (by 14%), and for the ratio (NAD + NADP)/(NAD • H_2 + NADP • H_2) to decrease. Meanwhile adrenalin, which excites both α- and β-adrenergic receptors, in a small dose (25 μg/kg) increased the content of both oxidized (by 24%) and reduced forms (by 48%) of pyridine nucleotides. The ratio (NAD + NADP)/(NAD • H_2 + NADP • H_2 showed a tendency to decrease on account of a greater increase in the content of reduced forms, indicating active function of the respiratory chain. A large increase in the dose of adrenalin (to 1000 μg/kg) was followed by a marked decrease in the content of oxidized forms (by 22.2%) and of the total pyridine nucleotides (by 18%). The ratio (NAD + NADP)/(NAD • H_2 + NADP • H_2) showed a tendency to diminish. Noradrenalin had no significant effect on the level of pyridine nucleotides for after a small dose the content of oxidized forms of nicotinamide coenzymes showed only a tendency to increase, and after a large dose a corresponding tendency to decrease.

Comparison of the action of catecholamines and methylxanthines on the content of pyridine nucleotides thus shows that the effect of methylxanthines is similar to the excitatory action of catecholamines on β-adrenergic receptors (the action of isoproterenol). These parallel changes suggest that the effects associated with excitation of β-adrenergic receptors are brought about through the participation of catecholamines, the level of which in the depots rises under the influence of methylxanthines.

LITERATURE CITED

1. A. M. Rusanov and G. P. Alekseeva, Farmakol. i Toksikol., No. 1, 74 (1967).
2. S. E. Severin, Abstracts of Scientific Proceedings of the Twenty-Sixth Session of the General Assembly of the Academy of Medical Sciences of the USSR [in Russian], Moscow (1967), p. 9.
3. N. O. Stepanyan and L. A. Tseitlin, Byull. Éksperim. Biol. i Med., No. 7, 51 (1968).
4. S. B. Frantsuzova, Byull. Éksperim. Biol. i Med., No. 5, 62 (1974).
5. R. A. Frol'kis, "Components of the tissue respiratory chain of the myocardium and their changes in myocardial infarction," Author's Abstract of Doctoral Dissertation, Khar'kov (1971).
6. A. G. Khalmuradov, Ukr. Biokhim. Zh., No. 3, 401 (1963).
7. L. A. Tseitlin, in: Progress in Biological Chemistry [in Russian], Vol. 8, Moscow (1967), p. 249.
8. B. Berkowitz and S. Spector, Europ. J. Pharmacol., 13, 193 (1971).
9. N. S. Dhalla, V. Balasubramanian, et al., Canad. J. Physiol. Pharmacol., 49, 302 (1971).
10. E. Glocky and P. McLean, Biochem. J., 61, 388 (1955).
11. J. W. Huff and W. A. Perlzweig, J. Biol. Chem., 167, 157 (1947).
12. B. L. Kennedy and S. Ellis, Arch. Internat. Pharmacodyn., 177, 390 (1969).
13. O. Strubelt, Arch. Internat. Pharmacodyn., 179, 215 (1969).
14. E. W. Sutherland and T. W. Rall, Pharmacol. Rev., 12, 265 (1960).
15. L. Triner and G. G. Nahas, C. R. Soc. Biol. (Paris), 160, 1905 (1967).