MECHANISM OF ACTION OF NAPHTHYLVINYLPYRIDINE

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Naphthylvinylpyridine (NVP) inhibited cholineacetyltransferase (CA) activity in the cat cerebral coretx (50 mg/kg) and mouse brain (100 and 250 mg/kg) but had no effect on acetyl- and butyrylcholinesterase activity or on the acetylcholine (AC) concentration in the mouse brain. In a dose of 25 mg/kg, NVP did not affect CA activity. After preliminary injection of NVP (25 and 250 mg/kg) the duration of hexobarbital sleep was considerably increased in mice, and in a dose of 250 mg/kg (but not 25 mg/kg) it increased the atropine activity in mice poisoned with armin*. In cats, NVP reduced AC liberation in the cerebral cortex occurring spontaneously and induced by atropine and electrical stimulation of the brain-stem reticular formation. It is suggested that the pharmacological effect of NVP, if given together with atropine and armin, is attributed chiefly to the anticholineacetyltransferase action and inhibition of synthesis of newly formed AC.

KEY WORDS: cholineacetyltransferase inhibitor; atropine; armin; potentiation.

Preparations of the styrylvinylpyridine group can inhibit cholineacetyltransferase (CA) and depress activity of microsomal enzymes (ME) [1, 5, 7]. Naphthylvinylpyridine (NVP) has been used most extensively in pharmacological research and has a significant effect on various forms of learning, behavior, and memory in experimental animals and also on the action of various psychotropic drugs [5-7]. However, it is not yet clear whether the pharmacological effect of NVP is connected with CA inhibition and lowering of the acetylcholine level in the brain or with inhibition of activity of the microsomal enzymes [7, 10].

The anticholineacetyltransferase action of NVP and its ability to inhibit ME activity was studied and the role of these factors was established during combined administration of the preparation with the cholinolytic and with an acetylcholinesterase (ACE) inhibitor.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 20-25 g and on cats weighing 3-5 kg. ME activity was assessed from the duration of sleep of the mice after intraperitoneal injection of hexobarbital (80 mg/kg). The toxicity of armin was determined in mice: 1) in conjunction with NVP given in a dose of 250 mg/kg, 2 and 5 h before armin, 2) in conjunction with atropine (4 mg/kg) 15 min before the armin, 3) in conjunction with a combination of NVP and atropine in the same doses and given at the same time, 4) in conjunction with the same combination but with NVP given in a dose of 25 mg/kg. The values of LD_{50} were calculated by the method of Litchfield and Wilcoxson, NVP and atropine were injected intraperitoneally, and armin was injected subcutaenously. CA activity was determined by Hebb's method [8] and ACE activity and butyryl-

TABLE 1. Duration of Hexobarbital Sleep in Mice Receiving NVP (M ± m)

Experimental conditions	Number of mice	Duration of sleep (in min)	P
Control (hexobarbital)	10	7±2	
NVP (250 mg/kg) + hexobarbital 2 h later	10	209±41	<0,001
NVP (250 mg/kg) + hexobarbital 5 h later	10	65±18	<0,01
NVP (25 mg/kg) + hexobarbital 2 h later	9	101±5	<0,01

*Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

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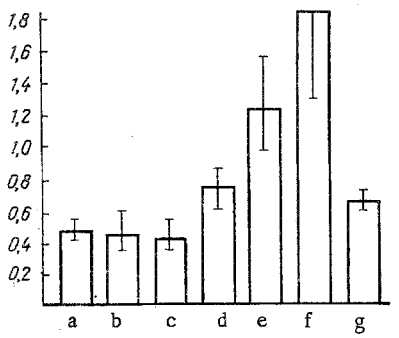

Fig. 1. Changes in LD_{50} of armin in mice after preliminary injection of NVP (250 and 25 mg/kg) and atropine (4 mg/kg): a) armin; b) NVP (250 mg/kg) + armin 2 h later; c) NVP (250 mg/kg) + armin 5 h later; d) atropine + armin 15 min later; e) NVP (250 mg/kg) + atropine 1 h 45 min later + armin 2 h later; f) NVP (250 mg/kg) + atropine 4 h 45 min later + armin 5 h later; g) NVP (25 mg/kg) + atropine 1 h 45 min later + armin 2 h later. Ordinate, LD_{50}.

cholinesterase (BCE) activity by Hestrin's method [9]. The content of free and bound acetylcholine in the mouse brain was studied by the method of Crossland and Slater [4] and liberation of acetylcholine by the method of MacIntosh and Oborin [2], modified for free behavior of the animals. Experiments were carried out on nine cats with a capsule introduced for superfusion of the cerebral cortex and with bipolar electrodes implanted into the brain-stem reticular formation (coordinates AP 0, L2, H-5). The reticular formation was stimulated for 10 min by means of a Nihon-Kohden electronic stimulator with series of square pulses (2 msec, 100 Hz, 2-7.5 V).

EXPERIMENTAL RESULTS AND DISCUSSION

NVP in a dose of 250 mg/kg significantly increased the duration of hexobarbital sleep in the mice (Table 1). The sleep was much more prolonged in mice receiving NVP 2 h before injection of the hexobarbital than in animals receiving NVP 5 h before hexobarbital. In a dose of 25 mg/kg, given 2 h before hexobarbital, NVP also significantly increased the duration of sleep in mice.

The value of LD_{50} for armin when given with NVP was unchanged (Fig. 1a, b, c), but it was greatly increased in animals receiving atropine 15 min before the armin (Fig. 1d). In mice receiving NVP together with atropine, LD_{50} of armin increased still more (Fig. 1e, f).

The results of investigation of the mouse brain CA activity showed inhibition of the enzyme by NVP to a degree that depended on the dose of the preparation (Table 2). Both in mice and in cats the inhibitory action of NVP was stronger 5 h than 2 h after its administration, and 2 and 5 h after injection of NVP (250 mg/kg) the ACE and BCE activity in the mouse brain was unchanged. The level of free and AC also was unchanged (Table 3).

The intensity of spontaneous liberation of acetylcholine in the cerebral cortex of intact cats was unchanged for 2 h. In the period of stimulation of the reticular formation the liberation of acetylcholine increased (Fig. 2a). Intramuscular injection of atropine (2 mg/kg) led to a marked and prolonged increase in acetyl-

TABLE 2. Effect of NVP on CA Activity of Mouse and Cat Brain

Species of animals, tissue investigated	Mode of injection of NVP	Dose of NVP (in mg/kg)	Time between injection of NVP and sacrifice of animal (in h)	Number of animals (control/experiment)	CA activity (in % of control)	P
Mice, brain without cerebellum	Intraperitoneally	25	2	9/9	78	>0,05
		100	2	7/7	60	<0,05
		250	2	7/8	50	<0,05
		250	5	9/9	38	<0,05
Cats, cerebral cotex	Intramuscularly	50	2	5/5	62	<0,05
		50	5	5/5	42	<0,05

TABLE 3. AC Content in Brain of Mice (in $\mu g/g$ wet weight of tissue) after Injection of NVP (250 mg/kg)

Experimental conditions	Bound AC (M ± m)	n	P	Bound AC (M ± m)	n	P
Control	0,92±0,08	9		1,47±0,12	13	
5 h after NVP	1,20±0,12	9	>0,05	1,25±0,10	13	>0,05

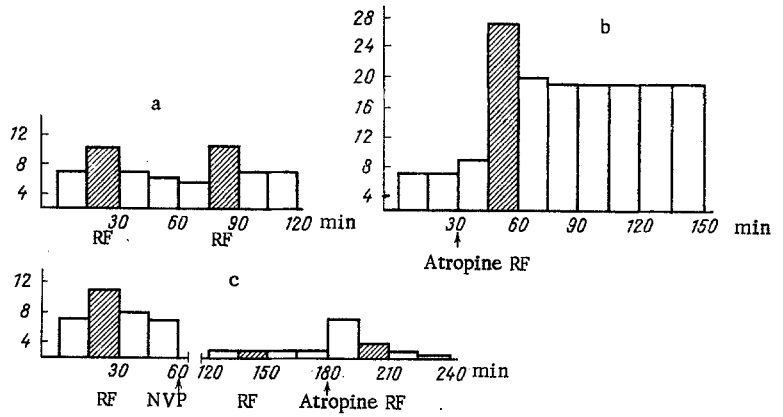

Fig. 2. Liberation of acetylcholine by cat cerebral cortex under free behavioral conditions: a) spontaneous and induced liberation in an intact cat; b) the same after intramuscular injection of atropine (2 mg/kg) and stimulation of reticular formation; c) the same after injection of NVP (250 mg/kg, intramuscularly) + atropine (2 mg/kg, intramuscularly) 2 h after NVP. Ordinate, quantity of AC (in ng) liberated by 1 cm^2 cerebral cortex in 15 min. RF) Stimulation of brain-stem reticular formation.

choline liberation, and this was intensified even further by stimulation of the reticular formation (Fig. 2b). Both spontaneous liberation of acetylcholine and its liberation induced by electrical stimulation were sharply reduced after 1.5-2 h in cats receiving NVP (250 mg/kg) (Fig. 2c).

The results of these investigations showed that the toxicity of armin was unchanged by NVP. However, the antagonism between atropine and armin was significantly strengthened by the action of NVP. It must be emphasized that the potentiating action of atropine was identical both 2 and 5 h after the injection of NVP. Meanwhile, the intensity of inhibition of ME by NVP was more than three times weaker 5 h and 2 h after its administration. This suggests that the potentiation of the atropine effect cannot be completely attributed to inhibition of ME and the corresponding inhibition of biotransformation of the compound, as stated by Goldberg et al. in their most recent paper [7]. The change in the effects of atropine when given after NVP correlate more closely with inhibition of CA activity. Preliminary administration of NVP in a dose of 25 mg/kg, not leading to any significant inhibition of CA, in conjunction with atropine did not increase the antagonism between the latter and armin, although injection of NVP in the same dose and at the same time caused definite inhibition of microsomal activity.

Despite its marked anticholineacetyltransferase effect, NVP did not lower the level of free and bound acetylcholine in the mouse brain. However, it sharply reduced the spontaneous and induced liberation of acetylcholine by the cat cerebral cortex. This evidently helped to maintain the normal level of the mediator at a time when its production was depressed. There is another possible explanation. In the modern view [3], the method of superfusion of the cerebral cortex adopted in the present experiments can give an estimate of the liberation of the liberation of newly synthesized AC. Presumably NVP inhibits the synthesis of precisely this fraction of the mediator. Although newly formed acetylcholine is regarded as being of great functional importance in cholinergic mediation, its contribution to the total content of tissue acetylcholine is small [5]. Inhibition of the synthesis of newly formed acetylcholine thus has little effect on the general level of mediator in the brain tissues. The increase in atropine activity when given in conjunction with NVP to mice poisoned with armin can probably be explained by depression of synthesis of the mediator and by a decrease in its liberation from presynaptic endings, for under those conditions the adsorption of the cholinolytic on the cholinergic brain structures must evidently be facilitated.

The results suggest that under the conditions of these experiments the pharmacological effect of NVP is connected with its anticholineacetyltransferase action and with inhibition of microsomal activity. However, the ability of NVP to depress CA activity and to inhibit the synthesis of newly formed acetylcholine is probably the most important factor.

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