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On the convulsive action of castrix

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SOME antagonists of vitamin B_6 are potent convulsant agents. Castrix(2-chloro-4-dimethylamino-6-methylpyrimidine) produces severe convulsions in mice which are protected by vitamin B_6.[1] So far nothing has been reported about the possible mechanism of action of this convulsant agent. To attempt a study of this mechanism, the effect of castrix on several enzymes, the relation between structural analogs of castrix and their toxic action, and the effects of several other agents on the convulsive action of castrix were studied.

Castrix was tested *in vitro* for the effect on the activities of three enzymes which require pyridoxal phosphate (PLP) as a cofactor, two enzymes which are involved in synthesis of PLP, and acetyl-

TABLE 1. EFFECT OF CASTRIX ON SEVERAL ENZYME ACTIVITIES

Enzymes	Control activity (100%)	Activity in the presence of castrix (% of control)		
		10^{-3}M	5×10^{-3}M	10^{-2}M
Glutamic decarboxylase	81 μl CO_2 evol./hr	86·5		66·3
Kynureninase (L-Kynurenine hydrolase EC 3.7.1.3)	0·55 μmole Kyn. split/hr	88·5		83·9
Glutamic oxaloacetic transaminase (L-Aspartate; 2-oxoglutarate aminotransferase EC 2.6.1.1)	0·79 μmole oxaloacetic acid formed/hr			100
PNP oxidase	0·1 μmole PNP oxidized/hr			106
PMP oxidase	0·084 μmole PMP oxidized/hr			106
PL kinase	0·06* μmole PLP formed/hr	95*	75*	41*
	0·087†			91†
Acetylcholinesterase				
mouse brain	19·6 μequiv. Ach hydrolyzed/hr		72	50
serum	20·8		31·5	3·5
serum + PLP 0·5 mM	16·6		45	0

* Concentration of PL was 10^{-4}M.

† 5×10^{-4}M.

Enzyme sources were as follows. Glutamic decarboxylase: supernatant of aqueous mice brain homogenate (1:10, w/v) at 20,000 *g* for 30 min. Kynureninase: supernatant of mice liver homogenate (1:2, w/v) in 0·14 M KCl at 20,000 *g* for 30 min, which was dialysed against 0·14 M KCl overnight. Glutamic oxaloacetic transaminase: supernatant of mice liver homogenate (1:15, w/v) in 0·1 M phosphate buffer of pH 7·4 at 18,000 *g* for 30 min, which was dialysed against the same buffer overnight. PNP or PMP oxidase: supernatant of mice liver homogenate (1:3·5, w/v) in 0·02 M phosphate buffer of pH 7·4 at 18,000 *g* for 30 min. PL kinase: purified enzyme from mice brain.[2] Acetylcholinesterase: mice brain homogenate in 0·9% NaCl or human serum. Enzyme activities were measured by the method described in a previous report[3] for glutamic decarboxylase, by the method of W. E. Knox[4] for kynureninase, by the method of H. U. Bergmeyer and E. Bernt[5] for glutamic oxaloacetic transaminase, by the method of H. Wada and E. E. Snell[6] for PNP or PMP oxidase, by the method of W. Pilz[7] for acetylcholinesterase and by the method described in a previous report[2] for PL kinase, respectively.

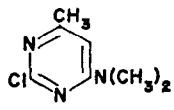
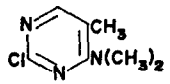
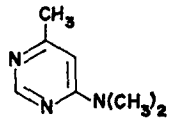
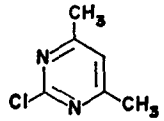
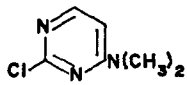
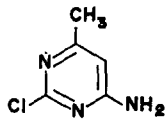
In all cases of glutamic decarboxylase, kynureninase and glutamic oxaloacetic transaminase, enzyme activities were determined without an addition of PLP.

cholinesterase(acetylcholine hydrolase, EC 3.1.1.7). As shown in Table 1, castrix in high concentrations inhibited the activities of pyridoxal(PL) kinase(ATP:pyridoxal 5-phosphotransferase, EC 2.7.1.35), glutamic decarboxylase(L-glutamate 1-carboxy-lyase, EC 4.1.1.15) and acetylcholinesterase. The inhibition of castrix on PL kinase was reversed by increased concentration of PL.

Castrix was tested *in vivo* for the effect on the activities of PL kinase, glutamic decarboxylase and acetylcholinesterase. In this study, 2·5 mg/kg of castrix was injected intraperitoneally and the mice (five mice in each group) were decapitated 40 min later. The supernatant of brain homogenate from control or from treated mice with castrix at 20,000 *g* for 30 min was used as the source of the enzyme. Enzyme activities were determined by the method described in the legend to Table 1. It was found that these enzyme activities were not significantly altered by convulsive doses of castrix.

Several structural analogs were tested in mice for their convulsive activity. The agents were injected intraperitoneally into DD male mice weighing 15–20 g. As shown in Table 2, castrix was the most potent convulsant of these analogs. 2-Chlor-4-dimethylamino-5-methylpyrimidine, 2-chloro-4,6-dimethylpyrimidine and 2-chloro-4-dimethylaminopyrimidine produced similar convulsions to castrix in mice; tonic–clonic seizures with running which were aggravated by sounds and ended with death from respiratory failure, dependent on dosage. These symptoms were prevented by vitamin B_6 injected intraperitoneally prior 30 min to these analogs. In addition to these symptoms, 2-chloro-4,6-dimethylpyrimidine caused mice extreme muscular weakness within 1 min and this symptom lasted for about 5 min. This symptom was not prevented by vitamin B_6. 2-Chloro-4-dimethylaminopyrimidine also had strong depressive action and the number of mice with convulsions over total number of

TABLE 2. TOXICITY OF SEVERAL STRUCTURAL ANALOGS OF CASTRIX

	Analogs*	Dose (mg/kg)	Conlvusion†	Death‡	Mean convulsive time (min)	Dose of pyridoxine (mg/kg)	Convulsion†	Death‡
(I)	 (Castrix)	2·5	5/5	5/5	57 ± 14	3 6	6/20 0/5	3/20 0/5
(II)		15	16/16	7/16	129 ± 37	18 36	4/6 0/5	2/6 0/5
(III)		160	5/5	5/5	0·41 ± 0·1	160	5/5	4/5
(IV)		200	5/5	5/5	112 ± 3	150	0/5	0/5
(V)		350	10/10	4/10	97 ± 22	150	0/5	0/5
(VI)		500	0/5	0/5	—	—	—	—

* Compounds II–VI were synthesized, respectively.[8–12]
castrix was kindly supplied by Mr. Umeda of Nihon Tokushu Noyaku Co.
† Number of mice with convulsions over total number of mice.
‡ Number of mice with death over total number of mice.

mice decreased in higher dosage (>350 mg/kg). The elimination of chlorine from castrix seemed to change the mode of convulsive action. Intense tremble and raised tail appeared within 1 min of severe tonic–clonic seizures without running. These symptoms were not prevented by vitamin B_6. Replacing the 4-dimethylamino group of castrix by amino group stopped the convulsive action but showed depressive action.

The structure of castrix resembles toxopyrimidine (TXP) which is a potent antagonist of vitamin B_6. Therefore, the effect of TXP on the convulsive action of castrix was assessed. TXP was found to have protective action against lethal convulsions of castrix as shown in Table 3. When TXP was injected intraperitoneally together with castrix (2·0 mg/kg), ED_{50} of TXP was 5·9 mg/kg. TXP at a dosage of 15 mg/kg given intraperitoneally prior 30 min to castrix and 30 or 40 min later, was found to have the same protective effects as TXP injected together with castrix.

TABLE 3. EFFECT OF TXP ON CONVULSIVE ACTIVITY OF CASTRIX

Dose of TXP (mg/kg)	Dose of Castrix (mg/kg)			
	0	2·0	2·5	5·0
	Convulsion-rate, % (Total number of mice)			
0		95 (45)	100 (5)	100 (5)
2		78 (9)		
5		53 (15)		
10		40 (5)		
15		10 (10)		100 (5)
50	0 (5)	0 (5)	0 (5)	60 (5)

Several compounds which are known to be convulsants or anticonvulsants were tested for their antidotal effects against castrix. As shown in Table 4 subtoxic doses of 4-deoxypyridoxine and semicarbazid were found to have protective effect. Isonicotinylhydrazine (INAH) was partially effective

TABLE 4. EFFECTS OF SEVERAL COMPOUNDS ON CONVULSIVE ACTION OF CASTRIX

Compound	Dose (mg/kg)		Injected time with respect to castrix (min)	Convulsion*	
	Castrix	Compound tested		Control group	Treated group with compound tested
INAH	2·5	50	0	12/12	3/12
4-Deoxypyridoxine	2·5	50	−30	7/7	0/7
Semicarbazid	2·5	50	0	5/5	0/5
DL-Penicillamine	2·0	50	−30	5/5	5/5
	2·0	50	0	5/5	5/5
	2·5	100	0	7/7	7/7
Thiosemicarbazid	2·0	4	0	5/5	5/5
Acetazolamide	2·5	10	0	5/5	1/5
	2·5	50	0	5/5	0/5
	2·5	50	+40	5/5	1/5
Metrazol	2·5	50	0	5/5	5/5
Atropine	2·5	10	0	4/5	5/5
Caffeine	2·5	50	0	15/15	4/15

* Number of mice with convulsions over total number of mice. All agents were injected i.p. into male DD mice weighing 15–20 g. Water was injected into control mice when compounds tested were injected into treated mice.

at a dosage of 50 mg/kg. Caffeine which was found to inhibit PL kinase *in vitro* (unpublished data) was partially effective at a dosage of 50 mg/kg. Acetazolamide which is inhibitor of carbonic anhydrase(Carbonate hydro-lyase, EC 4.2.1.1) and has anticonvulsant activity against epilepsy and convulsions induced by electrical stimulation,[13] had a protective effect against convulsions by castrix.

The mechanism whereby castrix competes with vitamin B_6 is uncertain. It was not explained by suggested mechanism for other B_6 antagonists.[cf. 14] Therefore, action of castrix may belong to different category. Such a compound may be a useful tool for elucidation of the role of vitamin B_6 functions in brain.

Department of Biochemistry,
Jikei University School of Medicine,
Minato-ku, Tokyo, Japan

YASUKO MURAKAMI
KENJI MURAKAMI
KATASHI MAKINO

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