The anticonvulsant effects of propranolol and β-adrenergic blockade[1]

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Summary. The anticonvulsant activity of racemic and (+)-propranolol was studied in rats. Neither drug changed the current to produce a minimal seizure in 50% of animals. Both drugs were effective in the maximal electroshock seizure test, the (+) isomer being more potent than the racemic form. Since the (+) isomer is practically devoid of β-adrenergic blocking activity, the anticonvulsant effects of propranolol do not result from β-adrenergic blockade.

The central nervous system (CNS) depressant and anticonvulsant effects of the β-adrenergic blocking drug propranolol have been repeatedly described[2-5]. The prevailing opinion that these effects are unrelated to β-adrenergic blockade is based mainly on the fact that the central effects occur at doses considerably higher than those required for effective β blockade and that not all β-blocking adrenergic drugs which reach the CNS show depressant activity[3]. To provide direct experimental evidence with respect to anticonvulsant activity, the effects of racemic propranolol on electrically induced seizures were compared with those of its (+) isomer. The (+) form of propranolol has less than 1/100 of β-adrenergic blocking activity of the (−) form but both isomers are equally potent as local anaesthetics[6].

Male Sprague-Dawley rats weighing 110–150 g were used. The animals were housed in groups of 5 and allowed free access to food and water except for the short period of testing. The ambient temperature was 22 °C. Anticonvulsant activity was determined in a maximal electroshock (MES) and minimal electroshock seizure threshold (EST-60 Hz) tests[7,8] with an apparatus designed by Woodbury and Davenport[9]. For MES test the rats were pretested and only animals exhibiting a tonic hindlimb extension were used[10]. ED_{50} to abolish the tonic hindlimb extension was determined by the probit method[11]. The seizure was characterized by durations of the initial tonic flexion and hindleg extension. In the EST test the current to produce a minimal seizure in 50% of the animals in the groups (CC_{50}) was determined by the 'staircase' procedure of Finney[12] and calculated by the probit method. Racemic propranolol and the (+) isomer were dissolved in physiological saline and injected in a constant volume of 0.8 ml i.p. 30 min before testing.

Both racemic and (+)-propranolol were effective in MES. The ED_{50} of the racemic compound was 4.5 mg/kg with 95% confidence limits from 3.4 to 6.0 mg/kg, that of the (+) isomer 2.6 (1.8–3.7) mg/kg. The 2 dose-response curves were parallel within the experimental error. The potency ratio was 1.7 (1.1–2.7), indicating that (+)-propranolol was more potent in MES test than the racemic compound.

After the administration of either isomer, the flexor component was prolonged, whereas the extensor one shortened. The duration of tonic flexion to tonic extension was increased in a dose related fashion (table). Neither racemic propranolol nor the (+) isomer in a dose of 3.0 mg/kg were effective in the EST test. CC_{50} was 15.2 (14.6–15.8) mA for the saline injected group, 16.0 (15.0–17.1) mA for the group treated with the racemic compound, and 16.1 (15.3–16.9) mA for the group treated with the (+) isomer. The CC_{50} ratios of the control and the treated groups were not significantly different from 1.

In accordance with previous findings[1,3,5] racemic propranolol was effective in our experiments in abolishing the tonic extension of the MES test. Further it decreased the severity of existing convulsions, as indicated by the increase of flexion to extension ratio[8]. No effect of propranolol was found on the seizure threshold, similarly as it was described in mice[5]. The drug appears therefore to have negligible, if any, effect on the initiation of the minimal neuronal discharge and the initial spread, while being effective in preventing the spread of seizure activity over the entire brain. The anticonvulsant profile of (+)-propranolol in the 2 tests was similar. Surprisingly, however, it was more potent in preventing the tonic extension phase of the MES test.

There is therefore little doubt that the anticonvulsant activity of propranolol does not result from β-adrenergic blockade. The same conclusion was reached with respect to the calming effects of propranolol in rats with septal lesions[4]. Further, the difference in potency in MES test between the racemic and (+) isomers possibly suggests that the presence of β-adrenergic blocking activities in the periphery or the CNS, tends to attenuate the anticonvulsant effects of propranolol. The underlying assumption for this suggestion, namely that the biological activities of the racemic and (+) form differ only with respect to β-adren-

Effect of racemic and (+)-propranolol on the pattern of maximal electroshock seizures (MES)

	Dose (mg/kg)	N	Flexion (sec)	Extension (sec)	F/E	Protected (%)
Control		44	3.4	8.5	0.40	0
Control saline injected		84	3.9	8.4	0.46	0
Racemic	2.0	12	4.6	5.5	0.8	8
	2.7	12	5.2	5.5	0.9	25
	3.5	14	6.4	3.0	2.1	43
	5.25	12	6.5	3.3	2.0	50
	7.7	12	8.7	0.8	8.7	83
(+)	1.0	13	5.4	6.1	0.9	15
	2.0	12	5.8	4.4	1.3	33
	3.5	13	8.8	2.1	4.2	62
	5.25	14	9.2	1.4	6.6	71
	6.0	8	9.7	0.7	13.9	88

N, number of animals. F/E, mean ratio of the duration of flexion to the duration of extension. Protected (%), percent of animals who did not exhibit the tonic extension.

ergic blockade, is likely but unwarranted. The finding that racemic propranolol is less potent as local anaesthetic than either (+) or (−) form suggests that such an assumption may not be universally applicable.

1 We wish to thank Hana Boucek for skilful technical assistance. Propranolol and (+)-propranolol were obtained through the courtesy of Ayerst Laboratories Ltd, in Montreal. This research was supported by the Medical Research Council of Canada, grant MA-4754.

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Effect of NPV of the armyworm *Mythimna (Pseudaletia) separata* on the silkworm *Bombyx mori*

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Summary. Nuclear polyhedrosis virus (NPV) material of the armyworm *Mythimna (Pseudaletia) separata* was administered in the form of PIBs and free virus rods to the larvae of silkworm *Bombyx mori*. Routes used for administration were topical, intrahaemocoelic and oral. The larvae were treated with following concentrations: 10×10^5 PIBs/L, 10×10^6 PIBs/L, 10×10^7 PIBs/L, 10×10^8 PIBs/L. In all the 3 experiments, the larvae showed neither any signs and symptoms, nor mortality due to polyhedrosis. Thus it appears that the NPV of *M. (P.) separata* is safe for the silkworm *B. mori*.

Totally 187 cross-transmissions of NPV among the insect species were attempted and 60 were successful[2]. Though NPV of 7 insect species were tested on the silkworm *Bombyx mori*, it was found to be innocuous[2].

The armyworm *Mythimna (Pseudaletia) separata* - a notorious agricultural pest - can be controlled by its own NPV in the laboratory and as well in the field[3]. However, the use of NPV on a large scale requires safety tests on beneficial insects like *Bombyx mori*. Since the effects of NPV of the armyworm *M. (P.) separata* on *B. mori* has not been investigated, the present experiments were conducted.

Materials and methods. Silkworm larvae reared on mulberry leaves were treated with the following concentrations higher than those required to infect the armyworm: 10×10^5 polyhedral inclusion bodies/larva, 10×10^6 PIBs/L, 10×10^7 PIBs/L and 10×10^8 PIBs/L. While in oral (experiment 1) and topical (experiment 2) treatments, at each concentration, 50 3rd instar larvae were used in 4 replications, during intrahemocoelic injection (experiment 3) treatment 40 5th instar larvae were replicated 5 times. In all the 3 experiments, controls generally received distilled water. However, in the 3rd experiment another set of control received viral suspension treated with alkaline solution ($NaCl+Na_2Co_3$) to free the viral rods from PIBs. Daily observations were made to determine the larval death due to NPV and other causes, and percent pupation and adult emergence.

Results and discussion. Results obtained from the 3 experiments could be summarized as follows. The treated larvae showed neither any signs and symptoms, nor mortality due to polyhedrosis. Survival rate of pupae and adults was 92.5-100%. Further, the treated larvae did not significantly differ from the controls in their intermoult period and larval duration. Hence it appears that NPV of the armyworm is non-infective to *B. mori*. In p.o. treatment (experiment 1) we also attempted to note the fate of the PIBs in the silkworm bodies by examining periodically the gut and faecal matter. Though PIBs could be found in the gut lumen but not in the faecal matter of the treated larvae after 4 h from the time of treatment, they were not detected either in the gut or faecal matter after 8 h and 24 h. The findings, therefore, suggest that, though the protein coat of PIBs is dissolved in the gut, the virus is noninfective to the silkworm *B. mori*. Such non-infectivity may be due to antiviral gut-juice which inactivates the viral material[4]. Since, in our work, even the free viral rods injected into the hemocoel failed to infect the silkworm, apparently factors other than gut-juice must be responsible for their innocuosness. In topical application, (experiment 2) when the exuviae of treated larvae were examined after the 3rd moult, PIBs were still found on their surface. This finding suggests that the polyhedral bodies have not penetrated the body wall of the silkworm.

Don Canerday[5], investigating the effect of high dosage level of cabbage looper NPV on some related Plusinae viz. *Pseudaoplusia includens, Rachiplusia ou* and *Angraplua biloba*, found that it was innocuous to these species when fed orally. Aruga et al.[6] when fed NPV of *Brathra brassicae* and *Hyphantria cunea* to *B. mori*, failed to produce any infectivity. Similarly Smith and Xeros[7] could not succeed in cross-transmitting the NPV of *Malacosoma alpica, M. disstria, M. americanum* and *M. pluviale* to *B. mori*. Our findings resembled these though we worked with different NPV, and we suggest that the NPV of the armyworm *M. (P.) separata* is safe for the silkworm *B. mori*.

1 Acknowledgments. We are grateful to the Karnatak University, Dharwad, for awarding UGC Junior Fellowship to S.G.D. and for financial assistance made available to S.B.M. We thank Professor M. Appaswamy Rao, Department of Zoology, for providing the necessary facilities. Our thanks are also due to Mr B. Chavannawar for supplying mulberry leaves and to the Government Silk Farm, Hindalaga, Belgaum, for providing silkworm eggs.

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