Neuromuscular blocking activity of dibekacin, a new semisynthetic aminoglycoside antibiotic

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Summary. Dibekacin possesses the untoward effect of producing a neuromuscular blockade. Neostigmine is unable to reverse the neuromuscular blockade produced by dibekacin. Calcium has not only the ability to restore the neuromuscular transmission but also to exert protective action against the neuromuscular blocking activity of dibekacin.

The newer members of the aminoglycoside antibiotic group play an important role in the area of antimicrobial chemotherapy because of their significantly broader antibacterial efficacy. Dibekacin[1] is a new semisynthetic aminoglycoside antibiotic with a potent antimicrobial activity against some *P. aeruginosa* strains resistant to high concentrations of gentamicin, amikacin and sisomicin[2]. The present paper reports the neuromuscular blocking activity of dibekacin and its interaction with calcium.

Material and methods. We used the isolated phrenic nerve hemidiaphragm preparation of the rat[3,4]. The phrenic nerve was stimulated with impulses from a square-wave stimulator. Supramaximal shocks of 4 V, 250 µsec at a frequency of $^1/_{15}$ sec were applied. We recorded the twitch tension of the muscle isotonically, using a force-displacement transducer, Harvard 357, on the paper of a polygraph (San-Ei 1103) with a speed of 2 mm/sec. We used standards of dibekacin sulfate and gentamicin sulfate and all the doses which are mentioned in the text are expressed in terms of the corresponding base of the antibiotic.

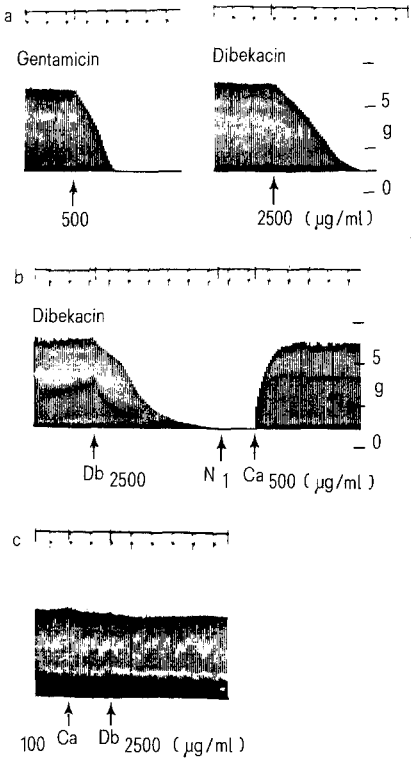

Fig. 1. Equivalent doses of dibekacin and gentamicin which produce a complete neuromuscular blockade at approximately the same time (a). The neuromuscular blockade produced by dibekacin is not reversed by neostigmine (N, 1 µg/ml) while it is reversed by calcium chloride, 500 µg/ml (b). Calcium chloride at a dose of 100 µg/ml exerts a protective action against the neuromuscular blocking activity of dibekacin when it is administered before the aminoglycoside antibiotic (c).

Results. Dibekacin, in a final bath concentration of 2500 µg/ml produced a complete neuromuscular blockade (figure 1, a). The equivalent dose of gentamicin, which produced a complete neuromuscular blockade under the same experimental conditions, was found to be 500 µg/ml (figure 1, a). The neuromuscular blockade produced by dibekacin is not reversed by neostigmine; it is reversed only by calcium (figure 1, b). Furthermore, calcium was found to exert a protective action against the neuromuscular blocking activity of dibekacin (figure 1, c). On the other hand, the neuromuscular blockade produced by ethylenediaminetetraacetic acid (EDTA) at a final bath concentration of 500 µg/ml had the same characteristics as that due to dibekacin, and in both cases the blockade was reversed by calcium (figure 2).

Discussion. Neuromuscular blockade is a recognized untoward effect of aminoglycoside antibiotics[5,6]. Our findings show that dibekacin, like other aminoglycoside antibiotics[7], possesses the untoward effect of producing neuromuscular blockade. The neuromuscular blocking potency of dibekacin appeared to be 5 time less than that of gentamicin. Neostigmine was found to be unable to reverse the neuromuscular blockade produced by dibekacin, while it is reversed only by calcium. Moreover, the neuromuscular blockade produced by EDTA had the same characteristics as that due to dibekacin and in both cases the blockade was reversed by calcium.

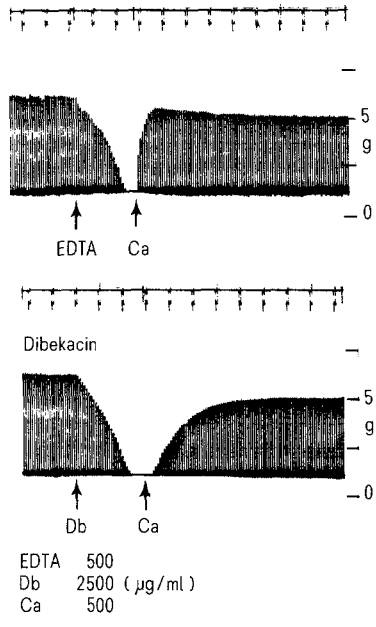

Fig. 2. Ethylenediaminetetraacetic acid (EDTA) at a final bath concentration of 500 µg/ml produces a complete neuromuscular blockade which is reversed by calcium chloride (500 µg/ml). This blockade has the same characteristics with that of dibekacin (2500 µg/ml) which is also reversed by calcium chloride (500 µg/ml).

According to our findings and those reported by other investigators[4,7,8], dibekacin does not produce a non-depolarizing blockade, because a non-depolarizing agent should be antagonized by neostigmine[9].

The inability of neostigmine to reverse the neuromuscular blockade produced by dibekacin suggests an impaired acetylcholine release from the motor end-plate in response to nerve impulses. On the other hand, the similarities of the blockades produced by EDTA and dibekacin, the ability of calcium to restore the neuromuscular transmission, as well as the protective action of calcium against the neuromuscular blocking activity of dibekacin, lead to the assumption that aminoglycoside antibiotics are involved in the process of acetylcholine release by nerve impulses, antagonizing calcium ions. The ability of calcium ions to antagonize the neuromuscular blocking activity of dibekacin is related to their ability to inactivate the antimicrobial action of aminoglycoside antibiotics[10].

The clinical importance of the neuromuscular blocking activity of the aminoglycoside antibiotics lies with the respiratory depression and/or prolonged apnoea which is faced when the above antibiotics are administered concomitantly with non-depolarizing muscle relaxant agents[5,6].

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Myotoxic phospholipases A from snake venom, *Pseudechis colletti*, producing myoglobinuria in mice

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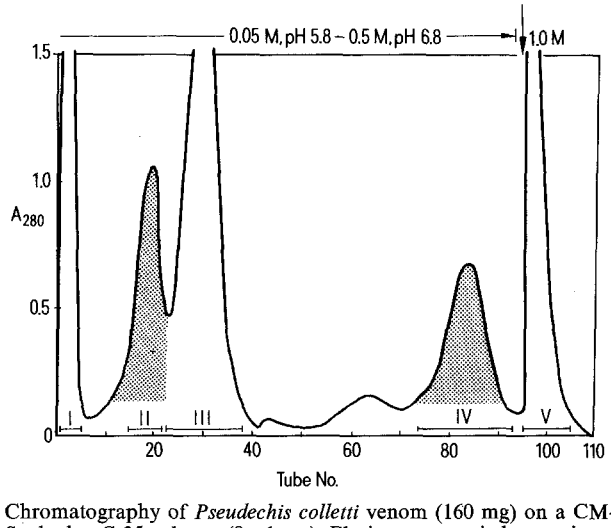
Summary. 2 proteins producing myoglobinuria in mice were isolated from the venom of the Australian elapid snake *Pseudechis colletti* and identified as phospholipases A showing close similarities in amino acid composition to a similarly acting enzyme from a sea snake venom *(Enhydrina schistosa)*.

Myoglobinuria is the most conspicious symptom in sea snake[1] *(Enhydrina schistosa)* as well as in several Australian Elapidae snake envenomations[2,3]; it has often been mistaken for haemoglobinuria. The active principle in sea snake venom producing this effect has been characterized as a basic phospholipase A[4]. This paper reports the isolation of 2 phospholipases A from an Australian snake venom, *Pseudechis colletti* which causes myoglobinuria in mice.

Materials and methods. The crude venom was purchased from Australian Venom Suppliers, Turramurra, NSW (Australia). Fractionation was performed by chromatography on CM-Sephadex C-25 using a linear gradient of 0.05 M ammonium acetate buffer pH 5.8 to 0.5 M, pH 6.8 for elution. Lethality of the fractions collected was assayed by s.c. injection into mice (4 male mice for each dose), myoglobinuria was observed by placing the mice on filter paper which was stained red by myoglobinuric urine. After elution of the paper with distilled water the extract was lyophilized; myoglobin was identified by gel filtration on a calibrated Sephadex G-75 column[5] (125 × 1.5 cm, 0.1 M ammonium acetate buffer pH 6.8) and by spectrophotometry before and after reduction using $Na_2S_2O_4$. Phospholipase A activity was determined according to the method of Marinetti[6] measuring the clearing of an egg yolk solution at 925 nm. Amino acid analysis was performed after 24, 48, 72 h hydrolysis of the protein samples in 5.7 N HCl at 110 °C; tryptophan was estimated after methane sulfonic acid hydrolysis for 24 h.

Results and discussion. By ion-exchange chromatography on CM-Sephadex C-25 the venom of *Pseudechis colletti* was separated into 5 main fractions. All exhibited high phospholipase A activity when tested on an egg yolk solution and all fractions, except I, showed lethal properties producing paralytic symptoms when injected s.c. into mice. But only fractions II and IV caused myoglobinuria after a lag period of 1–2 h; only myoglobin was found in the urine,



Chromatography of *Pseudechis colletti* venom (160 mg) on a CM-Sephadex-C-25 column (8 × 1 cm). Elution was carried out using a linear gradient of 0.05 M ammonium acetate buffer pH 5.8 to 0.5 M, pH 6.8 followed by stepwise elution with 1.0 M buffer. Fractions of 5 ml were collected at a flow rate of 30 ml/h. The dotted peaks (II and IV) produce myoglobinuria in mice.