The Physiological Effects of Ionic Lanthanum on the Insect Blood-Brain Barrier

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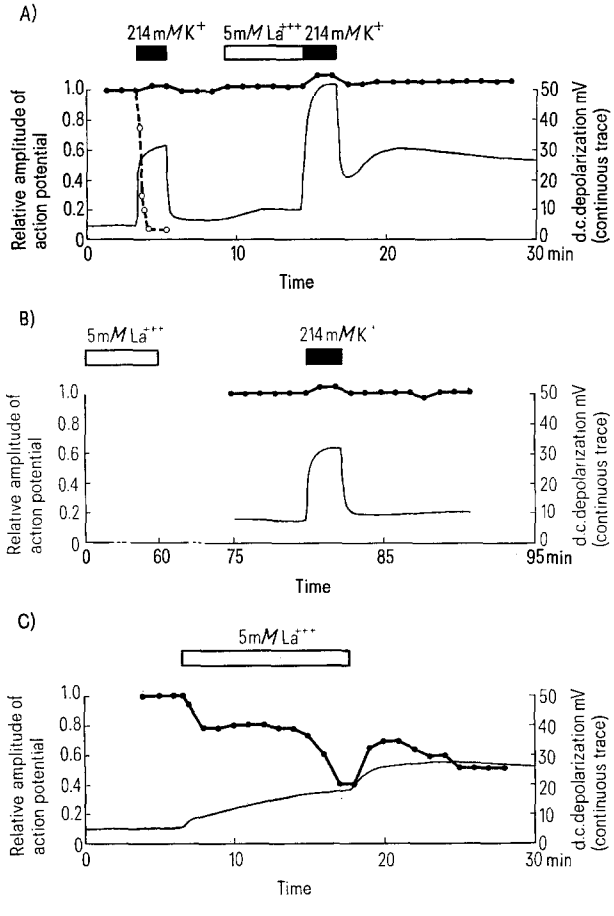
Summary. Lanthanum had a small effect on the barrier, but did not significantly increase its sodium or patossium permeability. There was no effect on nerve conduction unless the barrier was deliberately damaged. The results lend confidence to the use of lanthanum as an extracellular tracer.

The heavy metal lanthanum has been much used as an extracellular tracer substance, its small size and electron-opacity rendering it almost ideal for the purpose, although its ionic diameter (2.78 mm in a 2 m*M* solution[3]) is nevertheless rather larger than that of biologically important cations. Unfortunately, it has been reported to have adverse physiological effects on various tissues, at concentrations similar to those used in tracer studies (0.1–10 m*M*), including the frog neuromuscular junction[4], rabbit gallbladder[5] and squid giant axon[6]. We have been using colloidal and ionic lanthanum solutions as exogenously applied electron-opaque tracers, to investigate the permeability of the insect blood-brain barrier[7], and in view of the physiological effects of this substance, it was important to investigate its possible effect on the insect nerve cord. Although the ultrastructural studies ruled out the possibility of lanthanum damaging the barrier sufficiently to allow its own entry, the substance may, nevertheless, enable smaller substances to penetrate. This may be an important consideration, as 3.0 *M* urea has been reported to increase the permeability of the barrier to small cations, but no traversal of the barrier by lanthanum could be detected with the electron microscope[8]. The purpose of the present experiments was to investigate the effect of lanthanum on the cationic permeability of the barrier, as revealed by the electrophysiological effect of a high potassium, low sodium, saline; this saline has little effect on intact nerve cords, but causes a rapid conduction block in desheathed ones, apparently because the desheathing procedure secerely damages the under lying perineurium, which forms the barrier[9].



The effects of high-potassium and 5 m*M* lanthanum salines on the d.c. ('resting') and compound action potential amplitudes recorded from the cockroach abdominal nerve cord by the 'sucrose-gap' technique; details are given in the text. Compound action potential amplitudes were typically 20 mV. The vertical positioning of the d.c. traces is arbitrary.

The experiments were performed on abdominal nerve cords isolated from adult male cockroaches (*Periplaneta americana*). The experimental chamber was identical to that used in previous investigations[10]; it consisted of 5 parallel compartments, across which the nerve was laid, and allowed 'sucrose-gap' recordings to be made from the penultimate connectives. The normal saline contained 214 m*M* Na^+, 3.1 m*M* K^+, 1.8 m*M* Ca^{++}, 216.9 m*M* Cl^-, 0.2 m*M* $H_2PO_4^-$ and 1.8 m*M* HPO_4^{--}; in the test saline the sodium and potassium ion concentrations were reversed. The lanthanum saline additionally contained 5 m*M* La^{+++}, and the posphate buffer (which is precipated by lanthanum) was replaced by 0.3 m*M* *Tris* and 3.4 m*M* *Tris* chloride. All experiments were carried out at 24 ± 1 °C.

The effect of a 5 min exposure of the cockroach nerve cord to the lanthanum saline is shown in Figure A, which shows both the compound action potential amplitude (points) and d.c. 'resting' potential (continuous trace); the recording technique gives no reliable indication of the absolute value of the resting potential. The high-potassium saline causes a d.c. depolarization (which apparently occurs across the perineurium rather than across the axonal membranes[9]) and a slight increase in the amplitude

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of the compound action potential, whereas in a desheathed preparation (dotted lines) it causes a rapid conduction block. Exposure to the lanthanum saline does not abolish the barrier, as judged from the effect of the subsequent exposure to the high-potassium saline, although in this particular experiment there was some effect on the d.c. potential. In other experiments, however, the effect of the high-potassium saline was apparently normal even after a 1 h incubation in the lanthanum saline (Figure B). In none of these experiments did the lanthanum saline have any detectable effect on nervous conduction in the intact nerve cord, but it had severe and irreversible effects on conduction in desheathed nerve cords (Figure C).

The present experiments are thus in agreement with the ultrastructural observation that lanthanum cannot traverse the blood-brain barrier (the perineurium), but also show that it does not cause the barrier to become significantly more permeable to sodium or potassium ions. This substance does have some effect on the barrier, however, as judged by the complex effect on the d.c. potential (Figure A). The greater depolarization fo the d.c. potential in response to the second exposure to the high-potassium saline suggests that lanthanum decreases the sodium:potassium permeability ratio of the perineurium, although no estimate of absolute permeability changes has been made. By analogy with the effects of lanthanum on other tissues[4–6], it appears likely that there is an overall decrease in the ionic permeability of the perineurium as a result of exposure to lanthanum ions.

Thus, although ionic lanthanum is not totally without effect on the insect blood-brain barrier, it appears that the results of tracer studies using this substance at the low concentrations normally employed can be interpreted with some confidence.

The Effect of Cortisone on the Teratogenic Action of Acetylsalicylic Acid and Diphenylhydantoin in the Mouse

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Summary. Acetylsalicylic acid exerted a potentiating effect on cortisone – induced teratogenicity in the mouse. Diphenylhydantoin remained uneffective in this respect.

Cortisone, when given at a high dose to dams during the sensitive period of embryonic development, is known to produce cleft palate in the mouse foetus[1–15]. The failure of fusion of the embryonic palatal shelves in this species is known to be a non-specific effect occurring after the exposure of the dams to a variety of exogenous influences, including drugs of different chemical structure[16]. The occurrence of cleft palate is considered to result from a complex interaction between extrinsic and intrinsic factors responsible for an increase in maternal plasma corticosterone[17].

The present study was undertaken with a view to determining whether the administration of drugs of known teratogenic potential would alter the teratogenicity of cortisone in the mouse. Acetylsalicyclic acid (ASA) and 5,5-diphenyl-hydantoin (DPH) were selected because these drugs were shown by themselves to induce cleft palate in this species[18–27].

Materials and methods. Albino mice derived from an NMRI-strain and bred on our premises were used. Females aged 2 months were mated with males of proven fertility at the ratio of 1 ♂: 3 ♀♀. The day on which successful mating was verified by the presence of a vaginal plug was taken as 'Day 0' of gestation. Throughout the experiment, the females were kept 5 to a cage in an air-conditioned room at a temperature of 22 ± 0.5 °C and a humidity of 56 ± 3%. The room was illuminated for 12 h daily. A commercial standard diet was fed. Tap water was available ad libitum. The general condition of the dams was checked daily throughout the treatment. The dams were autopsied and the foetuses removed by Caesarean section on Day 18 of pregnancy. The foetuses were submitted to careful inspection with the aid of a dissection lens and were weighed individually.

ASA and DPH (Fluka Ltd., Buchs SG, Switzerland) were given orally by intubation from the 6th until the 15th day of pregnancy, inclusive. The doses were 500 mg/kg/day (ASA) and 100 mg/kg/day (DPH). A 2% aqueous

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