Technical Papers

A Possible Mechanism for the Nerve-blocking Action of *n*-Amyl Carbamate[1]

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This communication is a brief account of two experiments which suggest that in nerve block by *n*-amyl carbamate an interaction occurs which involves a sodium mechanism in nerve. The first experiment is summarized in Fig. 1. The sciatic-peroneal nerve of a

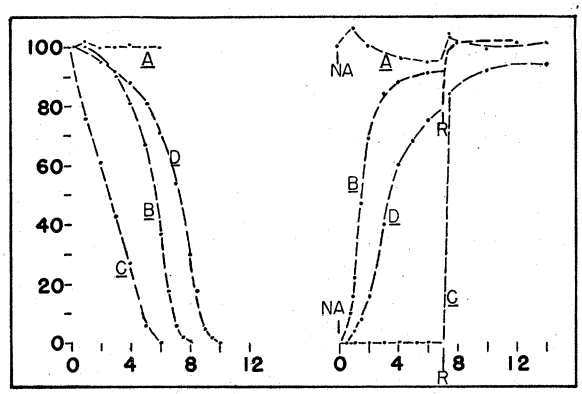

FIG. 1. Height of A potential, in percentage vs time in min. Curves on left represent action, curves on right are of recovery. *R* indicates replacement of test solution with Ringer's solution. *NA* indicates moment of addition of 0.011 *M* NaCl.

bullfrog was removed and desheathed. The nerve was then placed in a moist chamber and mounted on silver-silver chloride stimulating and recording electrodes. The monophasic action potentials in the A group of fibers were then recorded oscillographically. In Fig. 1 the compound A spike height, in percentage, is plotted against the time, in minutes, in order to illustrate the course of events. Curve *A* shows that *n*-amyl carbamate (0.0055 *M*) made up in Ringer's solution, when added to a 20-mm segment of nerve between the stimulating and recording electrodes, did not reduce the spike height in 6 min. At 7 min, which is the zero time (*NA*) in the recovery curve (*A*) to the right, a solution containing 0.011 *M* NaCl and 0.099 *M* choline chloride was added to the nerve segment in place of the amyl carbamate–Ringer's solution. This caused only slight reduction of the A spike. It is known (unpublished observations in this laboratory) that a 0.011 *M* NaCl is able to maintain activity in most of the A fibers of the bullfrog sciatic-peroneal nerve. Curve *B* next illustrates the point that a 0.11 *M* choline chloride solution, with no NaCl, caused a rapid conduction block which was complete in 8 min. This block was due to the absence of sodium ions. Rapid recovery from this block occurred when a solution of 0.011 *M* NaCl and 0.099 *M* choline chloride was added to the nerve segment at the time indicated as *NA*.

Curve *C* represents the significant section of the experiment. It shows that very rapid block was produced by a NaCl-free solution of 0.0055 *M* amyl carbamate in 0.11 *M* choline chloride. Thus a concentration of amyl carbamate, which in Ringer's solution was ineffective (Curve *A*), produced a facilitation of block when it was employed in the sodium-free solution. Upon the addition of a solution of 0.011 *M* NaCl and 0.099 *M* choline chloride in place of the amyl carbamate solution, no recovery took place until Ringer's fluid was added at the seventh minute of the recovery portion of Curve *C*. Complete inhibition of recovery by the ordinarily effective 0.011 *M* NaCl was obtained. The data for Curve *D* were next obtained. This was a repetition of the *B* section of the experiment and was carried out in order to show that the carbamate action was reversible and that the effect shown in Curve *C* was not due to a temporal deterioration of the nerve preparation.

The experiment of Fig. 1 illustrates an inhibitory action of *n*-amyl carbamate on the process of recovery by 0.011 *M* NaCl. It is also possible to demonstrate the converse effect—i.e., an inhibition of the amyl carbamate–blocking action by sodium ions. The effect is summarized in Fig. 2. Curve *A* illustrates the point that a 0.022 *M* NaCl solution, with sucrose serving as the osmotic substitute for the deficient NaCl, had no

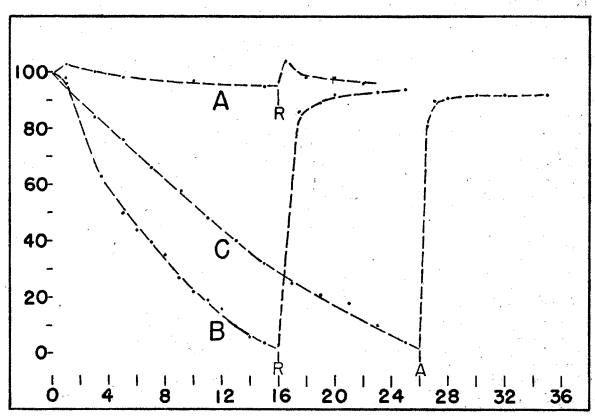

FIG. 2. Plot of A potential vs time (min). Curve *A*, lack of action by a solution of 0.022 *M* NaCl and 0.18 *M* sucrose. Curve *B*, block by a solution of amyl carbamate (0.0047 *M*), NaCl (0.022 *M*), and sucrose (0.18 *M*). Ringer's solution was added at moment indicated by *R*. Curve *C*, block by same test solution as in Curve *B*. At moment indicated by letter *A*, a solution of amyl carbamate (0.0047 *M*) and NaCl (0.11 *M*) replaced the test solution. Data for Curves *A* and *B* were obtained on one nerve, and Curve *C* data were obtained on the mate nerve. All solutions contained, besides the substances indicated, $CaCl_2$, KCl, and phosphate buffer as in Ringer's solution. The pH was 7.2–7.3, and the temperature of the bath for the nerve chamber was 23.5° C ± 0.5.

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significant action on the compound A spike of the desheathed sciatic-peroneal nerve of the bullfrog. Many experiments in this laboratory have shown that this concentration of NaCl is more than sufficient to maintain conduction in the A group of fibers in the bullfrog sciatic nerve. Curve *B* shows the rapid conduction block in the A fibers by a solution containing 0.0047 *M* amyl carbamate and 0.022 *M* NaCl. Addition of Ringer's solution at 16 min in place of the test solution caused a rapid return of the A activity. The crucial segment of the experiment is shown as Curve *C*. This illustrates the conduction block by a solution of 0.0047 *M* amyl carbamate and 0.022 *M* NaCl. At 26 min, when nearly complete block was achieved, the amyl carbamate solution was replaced by a second solution containing again 0.0047 *M* amyl carbamate but with the NaCl increased to 0.11 *M*. This caused a rapid recovery of conduction at a rate which was certainly no less than that of the previous run (Curve *B*). The experiment clearly demonstrates the reversal of block in the presence of amyl carbamate by increased sodium concentration.

Both types of experiments (Figs. 1 and 2) were simple, conclusive, and repeatable at will. The results suggest the existence of an intimate interaction between amyl carbamate and sodium. Present experiments, now in progress, suggest that other nerve-blocking drugs behave in a similar manner. The experiments, although subject to several interpretations, are most simply explained in the light of present knowledge on the assumption that amyl carbamate interferes, directly or indirectly, with a sodium mechanism. The experiments are of especial interest in relation to the sodium hypothesis of nerve conduction (*1*).

Reference

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The Action of Pectinol and Pectin Esterase on Sections of Rat and Guinea Pig Stomachs[1]

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McManus and Saunders (*1*) showed that periodic acid-Schiff (PAS) positive materials are removed with pectinase and less completely with pectinol. Pectin esterase did not remove these substances, but enhanced the PAS-coloration. The authors used human tissues (colon, bronchial mucins, and kidney) for their investigations. An attempt to compare the action of pectinol and pectin esterase on the stomach of the albino rat and of the guinea pig seemed of interest.

Ten 2–8-month-old nontreated rats of the Sprague-Dawley-Holtzman strain and 10 approximately 2-month-old nontreated guinea pigs (Rockland Farms) were killed by neckstroke. Their glandular stomachs were cut into 3 zones according to our standard procedure (*2*) and fixed in chilled ethanol, dehydrated, cleared in amyl acetate in the cold room at 4° C, and vacuum-embedded. Sections of 6μ thickness were mounted on slides with glycerine egg albumin, and left in a 50° C oven overnight. This step insured the adherence of the sections to the slides during incubation with the enzymes. The PAS-reaction (*3*) was carried out alone or after incubation of the sections with various enzymes.

The McManus reaction revealed PAS-positive fiber-like materials in the muscularis mucosae of the rat stomach. Those lying between the mucosa and the muscularis mucosae simulated a basement membrane. This was not observed in the guinea pig, in which PAS-positive materials were limited to the surface mucous cells.

Pectinol 100-D and Pectin Esterase No. 5 (Rohm and Haas) did not show any effect on the PAS-reaction at 1% strength and incubation for 30 min at 37° C. However, the same substances at 0.4% strength and incubation for 48 hr at 37° C, as recommended (*1*), showed the following results.

Pectinol 100-D incompletely removed the PAS-positive substances from the stomachs in accordance with previous findings (*1*). Furthermore, it brought out a positive PAS-reaction in parietal cells and erythrocytes that did not appear with the PAS-reaction alone or after saliva, ptyalin, or Taka-diastase incubations. Pectin esterase, also in accordance with McManus and Saunders (*1*), did not remove PAS-positive materials. It not only enhanced the coloration in the known PAS-positive constituents of the stomach, but caused a deep PAS-coloration in parietal and squamous cells, muscularis mucosae, submucosa, muscularis, and the walls of all blood vessels and erythrocytes. This was noted in the rat with greater intensity than in the guinea pig.

Incubation of rat stomach sections with Pectinol 100-D and Pectin esterase No. 5 at 37° C for 8 hr, followed by an accidental exposure to 60° C for 15 hr, gave the same results. Rat and guinea pig slides incubated with the same enzymes for only 8 hr at 37° C showed identical results. This demonstrated that 8 hr of incubation sufficed for the enzymatic action; furthermore, that a 15-hr incubation at 60° C did not affect the actions once they had taken place, although pectinases are rapidly inactivated at 60° C (*4, 5*).

After an 8-hr pectin esterase incubation, followed by a 15-hr pectinol incubation, less PAS-positive materials were observed than after pectin esterase incubation alone. This proved that pectinol removes some of the PAS-positive materials, whereas pectin esterase broke down PAS-negative mucoproteins into simpler PAS-positive cleavage products. Differences were noted here between the rat and the guinea pig. In the rat, less PAS-positive substances were removed by

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