plete time courses can be used rather than just the extrapolated initial rates. We are presently testing the practicality of this experimental technique, using an irreversible reaction with the stoichiometry A→P + Q.

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2 *A, B, P,* and *Q* are instantaneous concentrations of reactants and products; the subscripts o and e indicate initial and equilibrium concentrations respectively. K_e is the equilibrium constant; k_1 is the forward rate constant. ΔP is $P - P_o$. C is $-k_1/K_e$ for $A \rightleftharpoons P + Q$, k_1 for $A + B \rightleftharpoons P$, and $k_1(1 - 1/K_e)$ for $A + B \rightleftharpoons P + Q$. D is defined, and discussed at length, in the text.

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The effects of diltiazem on *Periplaneta americana*[1]

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Summary. The effects of the calcium antagonist diltiazem on nerve and muscle in the cockroach *Periplaneta americana* were examined. Diltiazem was observed to inhibit myogenic and glutamate-induced contractions of the visceral muscle while having either a potentiating, an inhibiting or a biphasic effect against proctolin-induced contractions. Against the isolated nervous system, diltiazem induced an increase in spontaneous discharge activity, followed by nerve block. Injection of diltiazem into cockroaches produced behavioral and toxic effects.

Diltiazem is a representative of a new class of pharmacological agents, the calcium antagonists. Recently, Ishida and Shinozaki[4] reported that diltiazem had differential effects on glutamate potentials and excitatory junctional potentials in an invertebrate preparation, the crayfish neuromuscular junction. In view of these interesting results, we examined the effects of diltiazem on neuromuscular transmission in visceral muscle of *Periplaneta americana* where there is evidence for both glutamate and proctolin as neurotransmitters and very few pharmacological tools to differentiate between them[5].

Materials and methods. Adult male *Periplaneta americana*[5] were used in all experiments. Bioassays using innervated and deganglionated hindguts were as previously described[6]. Hindgut neural stimulation was performed at 6–15 Hz applied in 2–10 sec trains at 30 sec intervals and the response quantified as reported earlier[5]. Diltiazem hydrochloride (active agent, 99.7% pure, m.p. 214 °C), from Nordic Laboratories Inc. Laval, Quebec, was added to the supply of saline perfusing the gut muscle to give a final concentration of 0.15, 0.3 or 0.6 mM. Bioresmethrin (92% pure) was from Wellcome Research Laboratories, Berkhamsted, U.K. Isolated nervous systems were obtained for recording by excising the ventral nerve cord with associated tracheae and pinning the nerve cord to the parawax floor of a recording chamber under saline that had been oxygenated for 30 min to 1 h. The trachea associated with the nerve cord were allowed to reach the surface of the saline as conduits for oxygen diffusion. Nerve cords consisted of the metathoracic and abdominal ganglia or in some experiments (where recordings were made from the proctodeal nerve) the IV, V and VI abdominal ganglia alone. Using a suction electrode, recordings were obtained from the proctodeal nerve where it joins the cercal nerve and from nerve 2A of the second abdominal ganglion. The signals were amplified through a W.P.I. DAM-6 differential amplifier, viewed on a Nicolet 2090-II digital storage oscilloscope, and recorded on a Phillips PM 8120 X-Y plotter. Toxicological studies were carried out by intrahemocoelic injections of 10 µl of diltiazem dissolved in cockroach saline, with the syringe introduced between the abdominal sclerites on the dorsal surface lateral to the heart. Treated cockroaches and controls were then placed on their backs in groups of 3–6 in glass beakers covered with muslin at 22–25 °C for observation of behavior and mortality effects.

Results. The cockroach hindgut preparation routinely displays spontaneous, apparently myogenic, contractions and responds with a sustained contracture to both glutamate and proctolin application[7]. The presence of diltiazem in the perfusion buffer at a concentration of 0.15–0.6 mM resulted in a marked reduction of spontaneous myogenic contractures within 1–2 min and changed the muscle response characteristics to glutamate and proctolin application (fig. 1 A, B). The response of the muscle lost its phasic character resulting in a tonic response resembling that produced by high potassium challenges (data not shown). The amplitude of these responses changed gradually before reaching stability after 16–30 min exposure of the preparation to diltiazem. Glutamate responses were inhibited in a dose-dependent manner (fig. 1C). The effect on proctolin responses was considerably more variable, the response observed (N = 18) being potentiation, inhibition, or potentiation followed by inhibition. These effects were reversible; continuous saline perfusion resulted in the recovery of the normal gut properties within a period of 4–6 min. Neurally evoked contractions were diminished in amplitude within 1 min and abolished within a period of 3–5 min by perfusion with 0.3 mM diltiazem. As this may be a direct effect of diltiazem on proctodeal nerve function, the action of diltiazem on spontaneous nerve activity in the isolated cockroach nervous system was investigated. When recordings were made from the severed distal end of the proctodeal nerve, spontaneous spike activity resembling that reported by Brown and Nagai[8] was observed. This firing pattern was stable for a period of over 1 h. Applications of diltiazem at final concentrations of 0.3–15 mM resulted in a transient increase in spontaneous firing activity followed by a decrease in firing frequency leading to nerve block (fig. 2A). This nerve block condition was seen in all proctodeal nerve recordings (N = 5) with onset occurring more rapidly at higher diltiazem concentrations. To test the generality of this effect,

recordings were also made from nerve 2A of abdominal ganglion II. Diltiazem at concentrations of 0.2–0.6 mM produced a marked increase in spontaneous activity in this nerve, including the appearance of bursts of spike activity (fig. 2B), followed by a decrease in nervous activity and apparent nerve block after 2–20 min (N = 4). Similar bursting discharges were seen following exposure to the pyrethroid insecticide, bioresmethrin (fig. 2C). Because of the similarities between the effects of diltiazem on nerve and muscle and the effects seen with insecticides on these same tissues[5,9], we investigated the in vivo toxicity of diltiazem to cockroaches. Topical applications of diltiazem in acetone to the ventral abdomen had no detectable effects. Intrahemocoelic injection of diltiazem, however, had marked effects on both insect behavior and survival. An injection of 10 µl 1.25 M diltiazem produced intoxication within minutes; the insects were lethargic and usually could not right themselves due to apparent paralysis of their hind legs in an extended position. These symptons of intoxication lasted over a period of 24 h and were accompanied by significant (75%, $P < 0.05$; N = 16) mortality at 48 h post-injection. At this dosage noticeable darkening of the insect abdomen was observed due to pigmentation in the midgut and other internal structures. Injection of 1.25 M diltiazem mixed with phenylthiourea (11 mM), a tyrosinase inhibitor[10], reduced this darkening without affecting intoxication or mortality. Frequently, diltiazem-treated cockroaches had bloated abdomens and less hemolymph could be obtained 24 h post-treatment than from saline injected controls. Injection of 10 µl 0.125 M diltiazem produced symptoms of intoxication lasting for a few hours, followed by a return to normal behavior and no mortality (N = 5). However, this con-

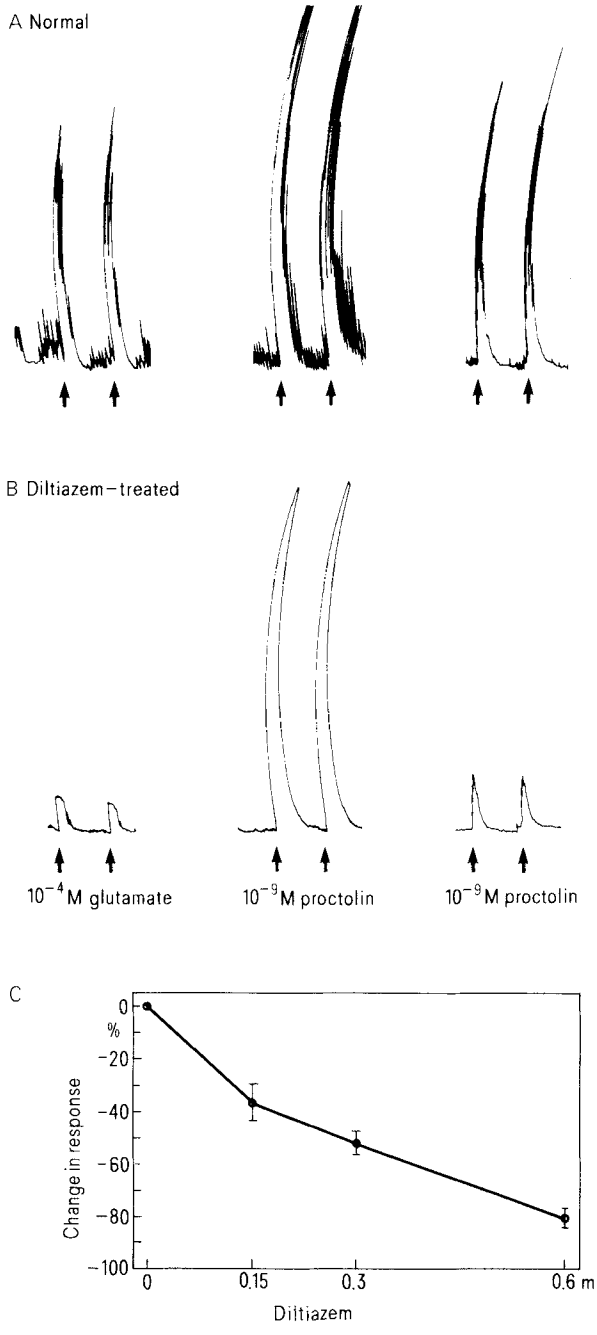

Figure 1. Effect of diltiazem perfusion on hindgut responses to 10^{-4} M glutamate and 10^{-9} M proctolin. Drugs applied for 20 sec at 2 min intervals. *A* Normal responses. *B* Responses after perfusion with 0.6 mM diltiazem for 22–30 min (see Results). Glutamate and the first proctolin responses were obtained with the same preparation. *C* Dose-response curve for inhibition of glutamate contractions by 0.15–0.6 mM diltiazem (N = 4 at each concentration).

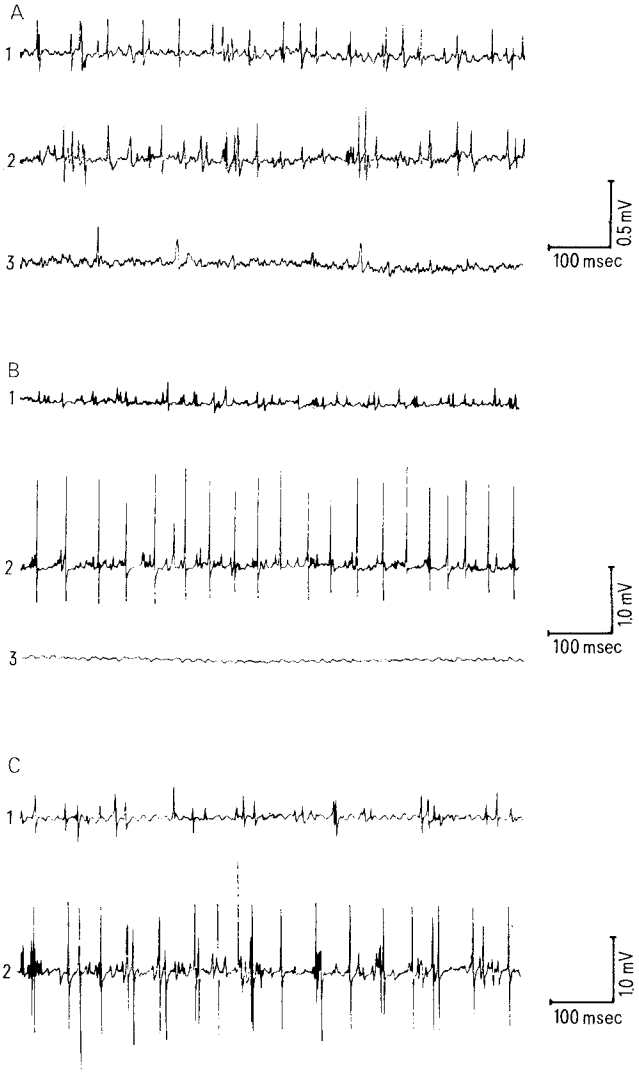

Figure 2. Effect of diltiazem and bioresmethrin on spontaneous nerve activity in the isolated nervous system. *A* Effect of 0.6 mM diltiazem on proctodeal nerve: 1) prior to diltiazem; 2) 14 min post-treatment; 3) 24 min post-treatment. *B* Effect of 0.2 mM diltiazem on abdominal nerve 2A: 1) prior to diltiazem; 2) 4 min post-treatment; 3) 10 min post-treatment. *C* Effect of 0.34 µM bioresmethrin on abdominal nerve 2A: 1) prior to treatment; 2) 3 min post-treatment.

centration of diltiazem injected into cockroaches deprived of water for 5 days resulted in significant (55%, $P < 0.05$; $N = 11$) mortality at 48 h post-treatment.

Discussion. Our observations that diltiazem inhibits spontaneous myogenicity in cockroach hindgut muscle and has inhibitory effects on glutamate responses extend the findings[4] on the crayfish neuromuscular preparation. In addition, diltiazem had varied effects against proctolin responses in this cockroach system. When tested on nerve preparations, diltiazem was found to have a biphasic effect on spontaneous neural activity, producing an initial excitation followed by inhibition of on-going activity. This inhibition or nerve block which was demonstrated to occur in the proctodeal nerve innervating the hindgut may account for the inhibition of neurally-evoked contractions seen with diltiazem perfusion of the hindgut preparation. The similarity between the effects of diltiazem and those of insecticides such as bioresmethrin (fig. 2B) on the nervous system led to an investigation of diltiazem toxicity in vivo. We found that diltiazem produced intoxication symptoms similar to those reported[11] by Van Asperen and Van Esch (paralysis, slow movements, animals lying on their backs) for injections of ethylenediamine tetraacetate (EDTA) solutions into cockroaches that reduced hemolymph free calcium to below detectable limits. Unlike EDTA, the calcium antagonist diltiazem produced longerlasting behavioral changes and significant mortality. Miller[9] reported that perfusion with low calcium concentrations duplicated the effect of pyrethroid poisoning on flight motor units in the housefly, and Clements and May[12] found that pyrethroids were able to inhibit glutamate- and neurally-induced contractions in locust muscle. The toxicity of injected diltiazem to cockroaches may be due to its effects on the nervous or neuroendocrine systems, with induced release of hormones perhaps contributing to toxicity[13]. The observed decrease of hemolymph levels in diltiazem poisoned cockroaches would be consistent with this idea since diuretic hormone is know to be released upon insecticide poisoning[14]. Interestingly, water-stressed cockroaches were more susceptible than unstressed controls to diltiazem poisoning.

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Examination of the fundus of the eye of renal hypertensive dogs

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Summary. Only 1 of 7 dogs with long-standing renovascular hypertension showed clear changes in the fundus. No distinct retinopathy was seen in the others. Ophthalmoscopy alone is thus of limited value in assessing the progress of benign hypertension in the dog.

In man, the following fundoscopic signs are of diagnostic and prognostic value in the management of hypertension: a) Sclerosis of the arterioles with segmental constrictions; b) Constriction of the venules at the intersections with the arterioles (Gunn's crossing sign); c) Oedema and exudation; d) Hemorrhages into the retinal tissue; e) Oedema of the optic disc.

As the dog is a frequently used experimental animal in cardiovascular research, the present investigation was made to see whether ophthalmoscopy shows comparable changes of the fundus in hypertensive dogs, and whether the severity of hypertension in the dog can be assessed in this way.

Methods. In a group of 14 mongrel dogs, comprising 7 with hypertension of 1–10 years' duration following bilateral constriction of the renal arteries[1] and 7 normotensive dogs of approximately the same age range (about 1 to over 10 years), the fundus was examined for evidence of vascular damage. Blood pressure was measured in a percutaneously punctured femoral artery at intervals of approximately 1 week. The fundus was photographed with a Kowa RC2 camera on Kodachrome film after administration of a mydriatic (tropicamide).

Results. a) Normotensive dogs. In 7 normotensive dogs between 1 and 13 years of age, the retina was found to differ from that of normotensive man in the following respects. The upper half of the retina appears in a luminous colour that varies from bright metallic blue to a golden yellow. This is due to a light-reflecting layer within the retina (tapetum lucidum) that improves night vision. The lower half of the retina has a dark pigmentation which, makes the vessels unrecognizable. The border-line of this pigmentation passes through or slightly below the optic disc. The myelination of the optic nerve fibers