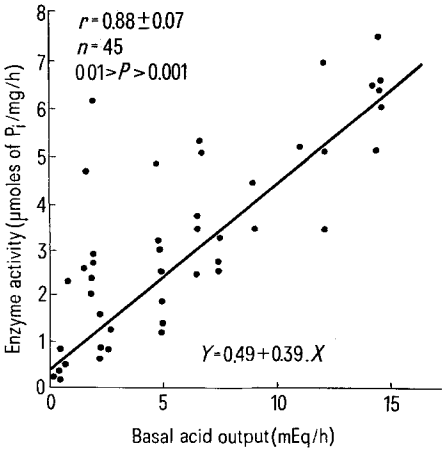

Correlation between the Na^+-K^+-dependent ATPase activity from fundic gastric mucosa (ordinate) and H^+ secretion (abscissa) in 45 patients (n). Each single point represents the average of three different measurements.

The Na^+-K^+-dependent ATPase activity was calculated as the difference between the total (obtained in presence of Mg^{2+}, Na^+ and K^+) and Mg^{2+}-dependent (obtained in presence of Mg^{2+}) alone[6-8].

Results and discussion. The Table shows the typical behaviour of membrane fractions from human gastric mucosa. The Figure indicates a positive and mathematically significant correlation between the H^+ secretion by the stomach and the magnitudes of Na^+-K^+-dependent ATPase activity from human fundic gastric mucosa. The biological importance of this positive and significant correlation is as yet unknown between the above-mentioned parameters of the stomach. The energy utilisation from ATP for H^+ secretion, by the way membrane ATPase system, is assumed by these results in the human fundic gastric mucosa.

Zusammenfassung. Es wurde bei Untersuchungen an 45 Patienten eine positive und mathematisch signifikante Korrelation zwischen der H^+ Sekretion und der Aktivitätsgrösse der ATPase von Membrane der Fundusschleimhaut gefunden.

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Inhibitory Effects of Dibutyryl and Cyclic AMP on the Compound Action Potential in the Frog (*Rana pipiens*) Sciatic Nerve

It has been reported that exogenous amounts of dibutyryl cyclic AMP and the methyl xanthines, theophylline and caffeine (which inhibit hydrolysis of cyclic AMP), were found to mimic the inhibitory effect of norepinephrine on Purkinje cells[1].

The cerebellar Purkinje cell study, like many others, reports the influence of certain pharmacological agents on the synaptic process without regard to the effects these compounds may have on membrane excitability. It should be kept in mind that drug modification of synaptic transmission may actually be a secondary outcome of the direct action of the compound on membrane excitability[2].

In view of this consideration, this paper reports the effects of dibutyryl and cyclic AMP, as well as, theophylline and caffeine on various parameters of the compound action potential in the peripheral nervous system (frog sciatic nerve).

Materials and methods. Frogs (*R. pipiens*) were obtained from Mogul-Ed Biological Supply Co. After double pithing, the sciatic nerve was carefully dissected in isotonic Amphibian Ringer's (240 mOsm). All fascia and overlying branches of the sciatic artery were carefully removed with fire polished glass dissecting needles. Each nerve was severed just distal to spinal ganglia 7, 8, and 9, and proximal to its bifurcation of peroneal tibial components. The preparation was then removed and placed on electrodes in a nerve chamber into which air was bubbled at approximately 0.5 cm^3 min.

The basic design of this study was to expose the nerve tract to solutions of a) Amphibian Ringer's during dissection; b) test solution for 30 min; c) Amphibian Ringer's for 30 min to measure recovery. Following each of the three exposures, nerve characteristics such as chronaxie, spike amplitude, latency and conduction speed were compared. The test solutions were various concentrations of dibutyryl and cyclic AMP. Caffeine (0.2 mM) and theophylline (4 mM) were tested alone or in addition to dibutyryl and cyclic AMP. All data were ob-

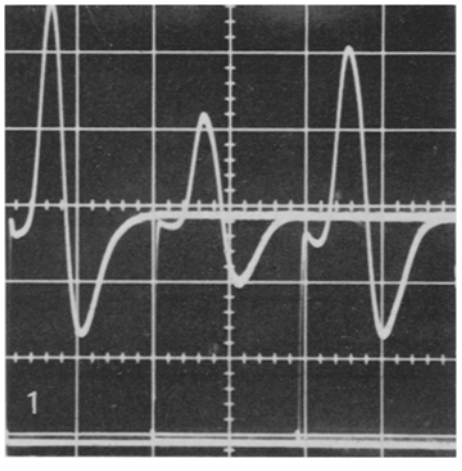

Fig. 1. First spike represents the inherent response to threshold stimulation. Second spike shows 14 mV decrease in response as result of 30 min exposure to 1×10^{-4} M cyclic AMP. Third spike indicates nearly complete recovery.

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served on a Tektronix 502A oscilloscope and permanently recorded by a Tektronix Polaroid camera.

Results. Six different concentrations (1×10^{-3} to 1×10^{-8}) of dibutyryl and cyclic AMP were compared as to their effects on the compound action potential, latency of response, and conduction speed. At each concentration, it was noted that both compounds decreased the spike amplitude. Greater effects (from 4–16 mV decrease) in spike height were observed with cyclic AMP, whereas, only a 2–6 mV decrease was noted in a comparable concentration of dibutytyl cyclic AMP. Following each drug test, all nerves washed for 30 min in isotonic Ringer's showed recovery within 10% of the initial measurements.

Strength duration curves were calculated for each concentration of dibutyryl and cyclic AMP. From these data, chronaxie values before and after drug exposure were compared for each nerve. It was noted that 1×10^{-4} *M* cyclic AMP increased the firing threshold more than any other concentration (Figure 1). This same concentration also increased latency by 0.1 m/sec, as well as, decreased conduction speed by 10 m/sec. All other levels of dibutyryl and cyclic AMP produced similar but smaller effects on the compound action potential.

The responses to dibutyryl and cyclic AMP were mimicked to varying degrees with theophylline (4 m*M*) and caffeine (0.2 m*M*). In 4 of 6 preparations when the nerve was washed in theophylline and cyclic AMP, a synergistic effect was noted; particularly in the spike amplitude (Figure 2). Caffeine (0.2 m*M*) with cyclic AMP was not as effective as theophylline and cyclic AMP.

In some preparations calcium-free Ringer's was used as the dissecting fluid and as the base solution containing cyclic AMP and the phosphodiesterase inhibitors. In all instances, it was found that when calcium was absent, the inhibitory effects of cyclic AMP were minimal. Specifically, calcium ions seemed to enhance the effects of cyclic AMP by increasing the membrane resistance 30% more than when calcium was absent.

Discussion. The data seem to indicate that a fibre tract separated from synaptic connections is affected by dibutyryl and cyclic AMP as well as, the phosphodiesterase inhibitors, theophylline and caffeine. All of these compounds caused a decrease in spike height, conduction speed and an increase in latency. These effects, however, were not linear with respect to increasing concentrations of dibutyryl or cyclic AMP. This observation may be explained by the structure of the frog sciatic nerve. In each fibre tract there are many nerve fibre of varying diameters, most of which are individually wrapped in a myelin sheath and further enveloped by a sleeve of endo and perineurium. These structural factors should all be considered as a possible explanation for the non-linearity between responses and different concentrations of the drug.

In view of these observations, one concentration (1×10^{-4} *M*) of cyclic AMP showed a greater effect than any of the others. While this level may be considered high, there were perhaps several dilution steps due the many membranous barriers before the solution finally reached the nerve fibre.

One of the more unusual findings in this study was the relative ineffectiveness of dibutyryl cyclic AMP as compared to cyclic AMP in causing a response. Dibutyryl cyclic AMP, because of its apparent ability to penetrate cell membranes more effectively than cyclic AMP[3], theoretically should cause a greater decrease in spike amplitude. This finding however, should not be entirely unexpected for such similar observations have been made in: a) the stimulation of renal gluconeogenesis[4]; b) inhibition of the cell growth[5]; and c) intestinal relaxation[6].

In many instances various studies have indicated a functional relationship between cyclic AMP and calcium ions: a) melanin-stimulating hormone action upon melanophores[7,8]; b) activation of phosphofructokinase in muscle[9]; c) effect of ACTH upon adrenal cortex[10]; d) the effects of vasopressin upon the toad bladder[11]. Primary to this study, the response of rat Purkinje cells to electrophoretically applied Ca^{2+} slowed the spontaneous discharge. In contrast to Ca^{2+}, metal chelators such as EDTA and citrate were capable of elevating discharge rates to the point of 'depolarization block', whereupon spike size decreased and rate of firing became rapid and abruptly stopped[12]. This association apparently holds true in the frog sciatic nerve where the combined influences of calcium and cyclic AMP were noted to decrease spike amplitude more efficiently than cyclic AMP alone.

Such decreases in response can be attributed to the effects of cyclic AMP on membrane permeability. In terms of ionic considerations which are primarily responsible for action potential formation, it may be suggested that cyclic AMP appears to decrease the membrane permeability to sodium ion influx and/or affecting the permeability by allowing an excess potassium ions to move outward. Both considerations could cause decreases in spike amplitude and seem in line with electrophoretic application of cyclic and dibutyryl AMP which mimick the effects of norepinephrine by producing hyperpolarizing responses in the Purkinje cells[12].

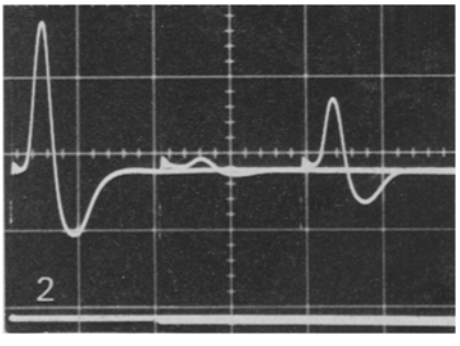

Fig. 2. Middle spike represents the synergistic effects of theophylline (4 m*M*) and cyclic AMP (1×10^{-4} *M*) in Amphibian Ringer's. Note the recovery spike (third) still shows the apparent effects of these 2 compounds.

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Résumé. Le dibutyryle et l'AMP cyclique ont apparemment un effet sur la conductibilité membraneuse. Cet effet se marque par une réduction d'amplitude et de vitesse de conductibilité aussi bien que par une augmentation du seuil à atteindre et du retard dans l'élaboration du processus. Ces effets ont été confirmés par la théophylline et la caféine. Les ions de calcium semblent accélérer les effets du AMP cyclique en augmentant la résistance membraneuse (réduction d'amplitude de l'impulsion nerveuse) de 30%.

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Somatotopic Organization of Inhibition in the Crayfish Abdomen of *Procambarus clarkii*

The discovery of identified neurons has made it possible to map the somatotopic organization of small invertebrate ganglia[1]. However no definite principles have been found to govern where a particular identified neuron has its soma located. The somatotopic organization of the neuromuscular system controlling the deep extensor and flexor muscles of the crayfisch abdomen have been studied. It is these muscles which serve to flex and extend the 'tail' during the swimming behavior which propels the animal backwards. All the motoneurons to the deep flexor muscles have been identified, and characterized both electrophysiologically and geometrically[2]. The present study reports both electrophysiological and morphological information regarding the peripheral inhibitor neuron to the deep extensor muscles, in the third abdominal ganglion of the crayfish.

Large *Procambarus clarkii* (5–8 inches) were used. A preparation consisting of a block of deep extensor muscles from 1 hemi-segment, including its intact motor nerve supply (NPM) and a section of the ventral nerve cord (VNC), was isolated in cold crayfish Ringer solution[3].

Antidromic stimuli of 0.1 msec duration were applied to NPM while individual somata within the 3rd abdominal ganglion were sampled one by one via the intracellular electrode. A deep extensor motoneuron soma (E1) located in this fashion was found in close proximity to F1, the flexor motor giant. The cell body is contralateral to the second root via which its axon exists the ganglion. A small (less than 15 mV) soma potential is recorded in response to NPM stimulation. The possibility of a chemical synapse between the NPM stimulating site and the cell body being monitored was ruled out when stimuli were delivered at rates of 100 Hz and above. No known chemical synapses to motor neurons are reported to follow at this high frequency[4]. The soma potential followed NPM stimulation 1:1 at this frequency. Next, depolarizing current pulses were passed through the intracellular soma electrode,

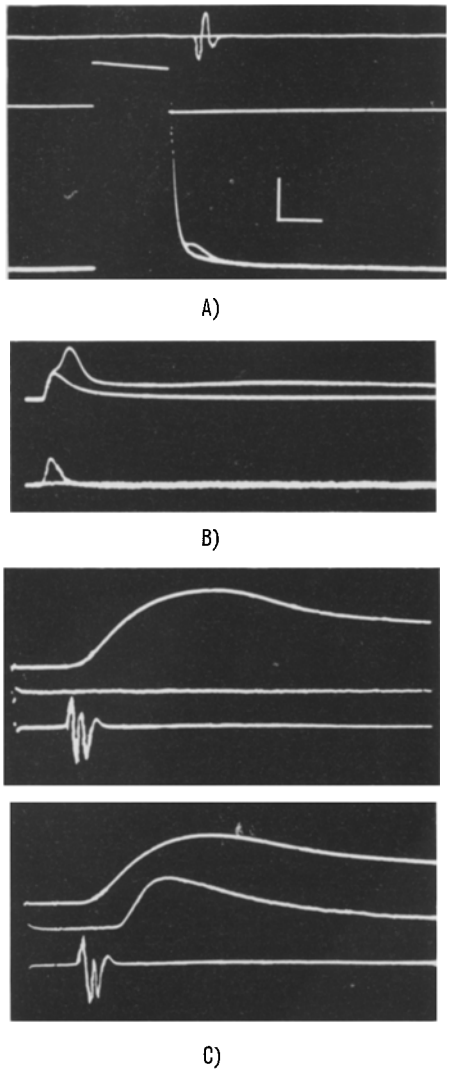

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Fig. 1. A) Depolarization of E1 via intrasomatic electrode. The top and bottom traces show 2 superimposed sweeps. The 1st sweep is subthreshold for E1 activation; the extracellular record of NPM (top trace) is flat while the intracellular potential (bottom trace) measured on a bridge circuit drops smoothly back to baseline. The 2nd sweep shows supra threshold stimulation of E1. An action potential is recorded at NPM, and a soma potential can be seen as a bump in the intrasomatic trace just before the return to baseline. The middle trace is the current monitor, recorded only during the suprathreshold sweep. Demonstration of inhibitory action of E1 on deep extensor muscles. B) 2 superimposed sweeps before and after activation of E1 by NPM stimulation. A 50% reduction of the muscle potential in a fibre of DEAM, the most medial deep extensor subunit (upper trace) occurs concurrently with E1 activity (bottom trace). C) Electrical activity in L2, the most lateral deep extensor subunit (top trace) before and after E1 activation (middle trace) via NPM stimulation. The repolarizations of L2 to 25 mV above resting potential after the peaks of the muscle potentials demonstrate the shortened time course of the excitatory junction potential current with E1 activity. The bottom traces are extracellular recording from proximal R2. Calibrations: A) 5 msec, 5 mV for bottom trace, for middle trace. B) 20 msec, 30 mV upper, 5 mV lower. C) 2 msec, 20 mV upper, 5 mV middle.