J. Physiol. (1977), **269**, *pp.* 753–766
With 6 text-figures
Printed in Great Britain

MODULATION OF CYCLIC NUCLEOTIDE LEVELS IN PERIPHERAL NERVE WITHOUT EFFECT ON RESTING OR COMPOUND ACTION POTENTIALS

BY JOHN P. HORN AND DONALD A. MCAFEE

From the Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33152, U.S.A.

(*Received 13 December 1976*)

SUMMARY

1. Cyclic nucleotide levels and compound action potential magnitudes were measured in frog sciatic nerves following exposure to carbachol, isoprenaline and cyclic nucleotide related substances.

2. The resting cyclic AMP level was 2·4 p-mole/mg protein and the cyclic GMP level was 0·27 p-mole/mg protein in desheathed nerves.

3. Isoprenaline (100 μM) caused a twofold increase in cyclic AMP without affecting cyclic GMP levels. Carbachol (100 μM) caused a twofold increase in cyclic GMP without affecting cyclic AMP levels.

4. The phosphodiesterase inhibitor theophylline (5 mM) augmented both cyclic AMP and cyclic GMP.

5. The magnitude of the resting or compound action potential was not affected by isoprenaline, carbachol, or phosphodiesterase inhibitors.

6. The cyclic nucleotides and their butyryl derivatives did not affect the magnitude of the resting or compound action potential, either when applied alone or in the presence of a phosphodiesterase inhibitor.

7. In contrast to synaptic tissue we conclude that hormone mediated cyclic nucleotide metabolism in peripheral nerve is unrelated to control of axonal excitability.

INTRODUCTION

There is considerable evidence to support the hypothesis that the action of certain neurotransmitters are mediated by cyclic nucleotides (Bloom, 1975; Greengard, 1976). For instance, adenosine 3′,5′-monophosphate (cyclic AMP) has been postulated to mediate the effects of noradrenaline on the β-adrenergic receptor (Robison, Butcher & Sutherland, 1971). Guanosine 3′,5′-monophosphate (cyclic GMP) has been postulated to mediate the action of acetylcholine at muscarinic cholinergic synapses and to produce a change in neuronal excitability antagonistic to the actions of the biogenic amines (Goldberg, O'Dea & Haddox, 1973).

It has been assumed by most investigators that the cyclic nucleotide related actions of neurotransmitters are confined to synaptic regions of the neurone. However, little attention has been given to peripheral nerve or other non-synaptic nervous tissue (McAfee & Greengard, 1972; Vande Berg, 1974; Kebabian, Steiner & Greengard, 1975). This paper describes the effects of neurotransmitter mimetics and cyclic nucleotide related substances on cyclic nucleotide content and action potentials in the frog sciatic nerve. We observed that certain neuro-transmitter mimetics can specifically alter cyclic nucleotide levels in the nerve. However, neither exogenous cyclic nucleotides nor the neuro-transmitter mimetics affect the compound action potential of this preparation. A preliminary report of some of these results has appeared (Horn & McAfee, 1976).

METHODS

Rana pipiens, averaging 25 g body weight were quickly decapitated, double pithed, skinned, and pinned to a dissecting tray. The sciatic nerve of each leg was sectioned distally and carefully dissected free from loose connective tissue, blood vessels, and nerve branches between the popliteal fossa and the lumbosacral plexus.

When indicated nerves were desheathed in Ringer solution (Feng & Liu, 1949) by girdling the connective tissue capsule and splitting the epineurium. Though not directly visualized, the presence of the epineurium was inferred by reflected light from the surface of the nerve and its breach by herniation of the superficial bundles of axons through the split. When completely girdled the epineurium and capsule were everted and peeled back over the surface of the nerve to each end.

The Ringer solution had the following composition (mM): NaCl, 73; KCl, 3; $NaHCO_3$, 35; $NaHPO_4$, 1·8; NaH_2PO_4, 0·59; $CaCl_2$, 2; dextrose, 16, and was equilibrated with 95 % O_2, 5 % CO_2. The pH of the solution was 7·4–7·6 at the temperature of the experiments (22–26° C) and was unaffected by any of the agents used in the experiments. The osmolarity of the Ringer as determined by freezing point depression was 230–240 m-osmole/l.

Extracellular recording. Sciatic nerves (3–4 cm) were secured by sutures to bipolar palladium electrodes in a covered Plexiglas nerve chamber (Harvard Apparatus) having a total volume of 10 ml. The distance between the stimulating cathode and the proximal recording electrode was 2 cm and the recording interelectrode distance was 1 cm. The portion of the nerve between the recording electrodes was crushed with forceps and ligated with 1–0 suture to provide monophasic (killed end) recording conditions. The nerve was immersed in Ringer solution which was continuously bubbled by 95 % O_2–5 % CO_2. At regular intervals (5 or 10 min) the bathing medium was withdrawn by suction and compound action potentials were generated at 1 Hz with the nerve suspended in the moist oxygenated atmosphere. Within 2 min measurements of compound action potential magnitude were obtained and normal Ringer or one modified by the addition of drugs was returned to the nerve chamber. Under these conditions the nerve remained viable with only a small decrement (about 10 %) in the magnitude of the compound action potential over several hours. The nerve was supramaximally stimulated by isolated rectangular pulses (50 μsec, 5 V), and the compound action potential conducted in myelinated axons and its electronic integral displayed on an oscilloscope (Fig. 1). Measurements of the amplitude of both the compound action potential and the integral were made by calipers from the face

of the oscilloscope (storage mode) or from photographic records. Variation in the recording interelectrode impedance during recording periods was minimized by suctioning dry the wall of the chamber between the recording electrodes. This procedure greatly reduced the fluctuation in recorded amplitudes caused by electrolyte short circuiting. Another source of error was the occasional failure to completely maintain monophasic recording conditions during the course of the experiment. Attenuation of the duration of the 'monophasic' signal derived from the proximal recording electrode could occur if a significant amount of activity appeared at the distal recording electrode. This results in an underestimate of the integral rather than the amplitude of the compound action potential.

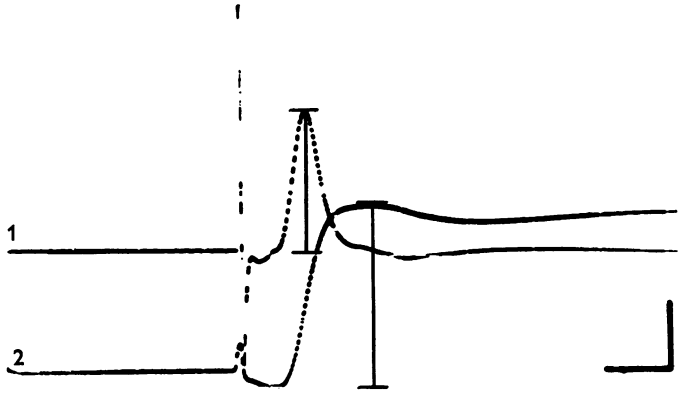

Fig. 1. A typical record of the compound action potential (trace 1) and its integral (trace 2) elicited in response to supramaximal stimulation of the frog sciatic nerve. The vertical bars in each trace indicate the position and amplitude of the two parameters measured in these experiments. The compound action potential was detected by a differential amplifier (2–10 kHz). The integral was derived by an operational amplifier with capacitive feed-back (time constant = 0·5 msec) that was directly coupled to the output of the differential amplifier. The calibration bars indicate 5 mV, 2·5 μV sec, and 1 msec.

Sucrose gap recording. In a few experiments the sucrose gap technique was employed using a Plexiglas chamber (Kosterlitz, Lees & Wallis, 1968; McAfee & Greengard, 1972). The advantages of this approach were that changes in resting membrane potential could be detected, the compound action potential remained monophasic and could be continuously monitored, and the short circuiting impedance ($\sim$1 MΩ) was stabilized. The potential across the gap was detected by calomel electrodes with a differential electrometer and displayed on an oscilloscope (DC-10 kHz) and chart recorder (DC-2 Hz). One side of the gap was always perfused (0·2–0·3 ml./min) with drug-free Ringer and the other side with drug-containing Ringer during the experimental period. The sucrose solution had an osmolarity of 240 m-osmole/l.

Cyclic nucleotide levels. Nerves were isolated and desheathed as before and then divided into proximal and distal halves. Thus each frog leg provided two lengths of nerve which were paired as control and experimental tissue. After a 60 min preincubation during which the nerve pairs were continuously superfused with normal oxygenated Ringer, one member of each pair (control nerve) was removed and homogenized in 250 μl. ice-cold conc. HCl-absolute ethanol mixed 1:60 (w/w). The

treated nerve was exposed to neurotransmitter mimetics for specific periods of time, removed and homogenized in the same manner as the control nerve. In some cases both members of the nerve pair were exposed to selective transmitter receptor antagonists or phosphodiesterase inhibitors for 30 min. Following this pretreatment period one member of the pair was removed and homogenized (control nerve) and the other exposed to a neurotransmitter receptor agonist (experimental nerve).

The homogenates were centrifuged at low speed, the supernatant separated and the precipitate containing the protein assayed (Lowry, Rosebrough, Farr & Randall, 1951) after dissolution with 1 N-NaOH. The supernatant was dried down in air at 70° C and redissolved in 0·5 M acetate buffer pH 6·2. Aliquots of this material were assayed for cyclic AMP and cyclic GMP by a modification of a commercially available radioimmunoassay (Steiner, 1973). The modification consisted principally of a reduced incubation volume (150 μl.) and a shortened, 3 hr, incubation period (non-equilibrium assay).

Chemicals and sources. Bovine serum albumin and Folin's reagent used for the protein assay were purchased from Sigma, St Louis, Mo, U.S.A. Theophylline and reagents for the cyclic AMP and cyclic GMP radioimmunoassays were purchased from Schwarz Mann, Orangeburg, N.Y. Adenosine-3′,5′-monophosphoric acid (cyclic AMP), and N^6-monobutyryl-adenosine-3′,5′-monophosphate (mbc AMP), guanosine-3′,5′-monophosphate (cyclic GMP) and, N^2-2′0-dibutyryl-guanosine-3′,5′-monophosphate (dbc GMP), as monosodium salts were purchased from both Sigma and Boehringer Mannheim. Caffeine, carbamylcholine chloride (carbachol), D,L-isoprenaline HCl, procaine as the free base, atropine sulphate, and tetrodotoxin were obtained from Sigma. RO 20-1724 and MJ 1999 (Sotalol-HCL) were generous gifts of Hoffmann-La Roche and Mead Johnson respectively.

RESULTS

Effects of exogenous neurotransmitter mimetics

Cyclic nucleotide levels. The endogenous level of cyclic AMP in 105 untreated desheathed nerves was $2{\cdot}4 \pm 0{\cdot}1$ (S.E. of mean) p-mole/mg protein. In eighty-seven nerves the cyclic GMP level was $0{\cdot}27 \pm 0{\cdot}02$ (S.E. of mean) p-mole/mg protein or about tenfold less than cyclic AMP. In paired studies there was no significant difference in the cyclic nucleotide content of proximal lengths of sciatic nerve as compared to distal lengths or between nerves from the right side as compared to nerves from the left side of the animal. The variance in cyclic nucleotide content of the sciatic nerve between animals was greater than the variance within an individual animal which justifies the paired experimental design.

The effect of isoprenaline and carbachol on cyclic nucleotide levels is presented in Fig. 2. Isoprenaline augments cyclic AMP to maximal levels (twofold) within 2 min but does not significantly affect cyclic GMP levels. Carbachol augments cyclic GMP to maximal levels (twofold) within 2 min but does not significantly affect cyclic AMP levels.

The ability of isoprenaline to augment cyclic AMP in the sciatic nerve was blocked by the β-adrenergic antagonist MJ 1999 and sustained in the presence of the phosphodiesterase inhibitor, theophylline (Fig. 3). The

antagonist MJ 1999 had no effect on cyclic nucleotide levels (data not shown), but theophylline augmented both cyclic AMP and cyclic GMP.

Isoprenaline was not effective in augmenting cyclic AMP levels in sheaths collected from the nerves used in this experiment. The endogenous levels of cyclic AMP in the nerve sheath ($1{\cdot}9 \pm 0{\cdot}2$ p-mole/mg

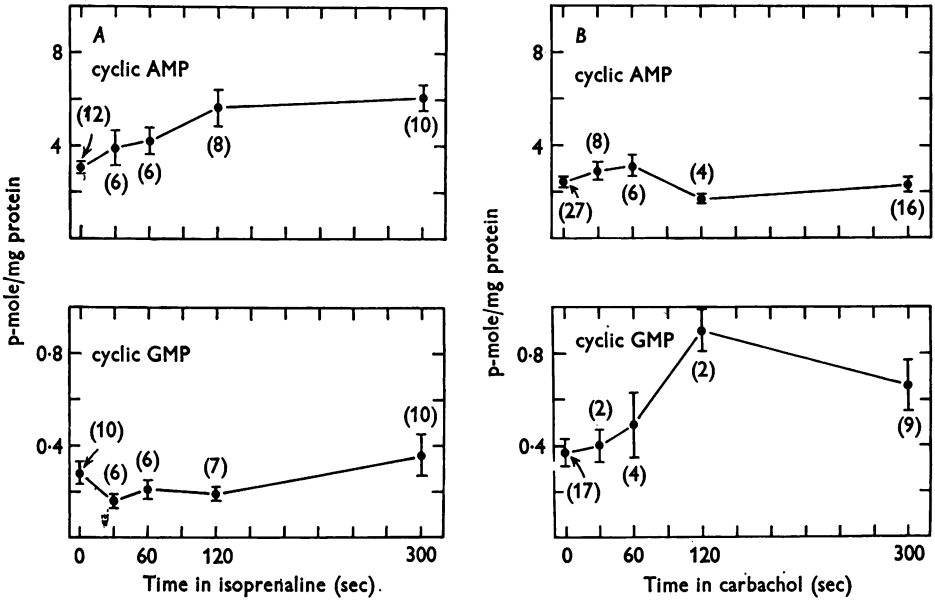

Fig. 2. Cyclic nucleotide levels in sciatic nerve following exposure to neurotransmitter mimetics for various periods of time. Desheathed frog sciatic nerves were superfused with Ringer containing 100 μM isoprenaline (part *A*) or 100 μM carbachol (part *B*) and then analysed for cyclic AMP, cyclic GMP and protein. Data are plotted as the mean $\pm$ S.E. of mean for the number of nerves in parentheses. In part *A*, cyclic AMP levels are significantly elevated by isoprenaline ($P < 0{\cdot}05$, Student's *t* test) at 60 sec and thereafter. In part *B*, cyclic GMP levels are significantly elevated by carbachol ($P < 0{\cdot}05$, Student's *t* test) at 300 sec.

protein) was nearly the same as that of axonal tissue. The small quantity of this connective tissue precluded measurements of cyclic GMP.

The ability of carbachol to augment cyclic GMP levels was blocked by atropine (Fig. 4). While theophylline during 30 min significantly elevated cyclic GMP (Fig. 3), carbachol was unable to further augment cyclic GMP when added to the bathing medium during the last 5 min of exposure to theophylline (Fig. 4).

Electrical stimulation (10 Hz, 5 min) of the sciatic nerve produced no statistically significant change in cyclic nucleotide levels. However, a

high K^+ (76 mM) Ringer solution in which KCl was substituted for NaCl generated a significant increase in cyclic GMP. In sucrose gap experiments this concentration of potassium shifted the gap potential 40 mV in the depolarizing direction. This increase in cyclic GMP level was not prevented by atropine at a concentration which blocked the increase caused by carbachol.

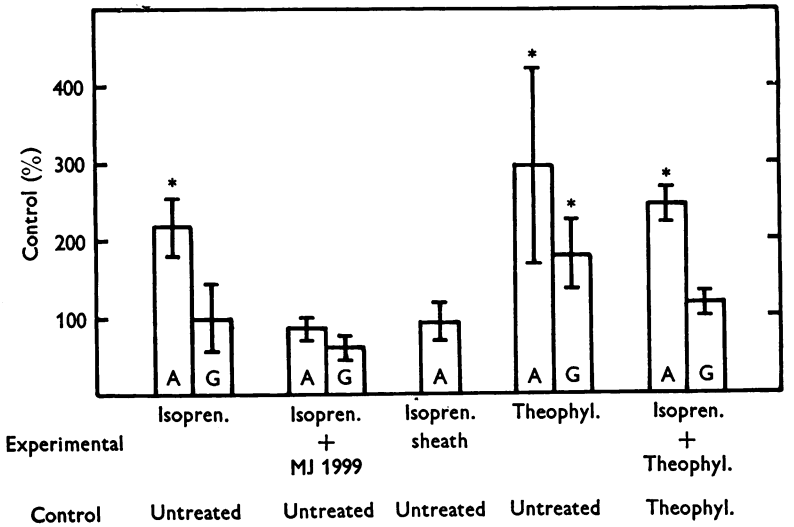

Fig. 3. The cyclic nucleotide content as % of control in paired sciatic nerves or nerve sheaths following exposure to isoprenaline and certain antagonists. Each bar is a measure (± S.E. of mean) of cyclic AMP (A) or cyclic GMP (G) content in experimental tissue relative to control. Nerves or nerve sheaths were exposed to 100 μM isoprenaline for 5 min and analysed for cyclic nucleotide and protein content. Some nerves were exposed to MJ 1999 (200 μM) for 20 min or to theophylline (5 mM) for 30 min. Isoprenaline was present for the last 5 min of the incubation as indicated. In these experiments four to seven tissue pairs were statistically evaluated by the paired t test. The asterisks indicate $P < 0{\cdot}05$.

Resting and compound action potential. Both of the neurotransmitter mimetics used in this study are known to affect cyclic nucleotide levels and excitability of synaptic tissues. The data in Fig. 5, drawn from a single sucrose gap experiment, is representative of three experiments in which compound action potentials and changes in the resting potential were monitored during exposure to Ringer containing carbachol and isoprenaline. However, in these experiments neither agent, at doses which augment cyclic nucleotides, caused any change in the magnitude of the compound action potential or resting membrane potential of desheathed sciatic nerve. Procaine and 76 mM-K^+ Ringer (data not shown) did re-

duce both the compound action potential and the resting membrane potential proving that changes in these parameters were detectable in our measuring system.

Effect of exogenous cyclic nucleotides

Exogenous cyclic nucleotides have been shown to affect the membrane potential and discharge frequency of neurones in synaptic tissue (Bloom, 1975). We tested the effects of cyclic nucleotides and related substances

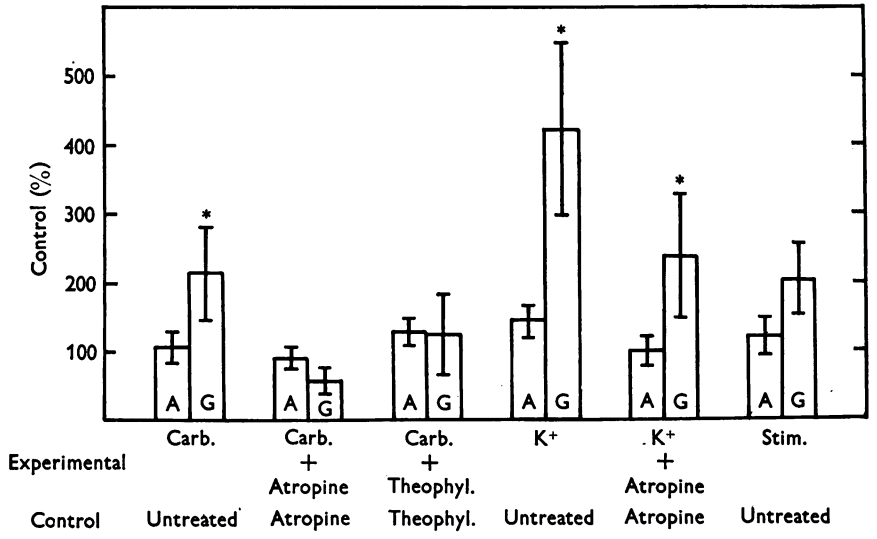

Fig. 4. The cyclic nucleotide content as % of control in paired sciatic nerves following exposure to carbachol, high K^+, or electrical stimulation. Each bar is a measure (± S.E. of mean) of cyclic AMP (A) or cyclic GMP (G) content in experimental tissue relative to control. In some experiments one member of each nerve pair was exposed to the various agents while the other served as the untreated control. Experimental nerves were exposed to 100 μM carbachol, 76 mM-K^+ Ringer, or electrical stimulation (10/sec) for 5 min, and analysed for cyclic nucleotide and protein content. In the other experiments both members of the pair were preincubated with atropine (25 μM) or theophylline (5 mM) for 30 min, one member (control nerve) removed and the other exposed to these antagonists with carbachol (100 μM) or high K^+ Ringer for an additional 5 min. In these experiments four to fourteen pairs were statistically evaluated by the paired t test. The asterisks indicate $P < 0{\cdot}05$.

on the compound action and resting potentials of frog sciatic nerves (Table 1, Fig. 6).

Sheathed nerves. Exposure to 0·1–1 mM concentrations of cyclic AMP, cyclic GMP, and their butyryl derivatives for 30 min did not change the magnitude of the compound action potential of sciatic nerves with the

sheath and epineurium intact (see Table 1). The phosphodiesterase inhibitors caffeine, RO 20-1724, and theophylline were without effect on the compound action potential. Under these conditions procaine and tetrodotoxin reversibly decreased the amplitude of the compound action potential 20–30%. During a 10 min exposure, procaine only desynchronized the compound action potential since the amplitude but not the integral was depressed.

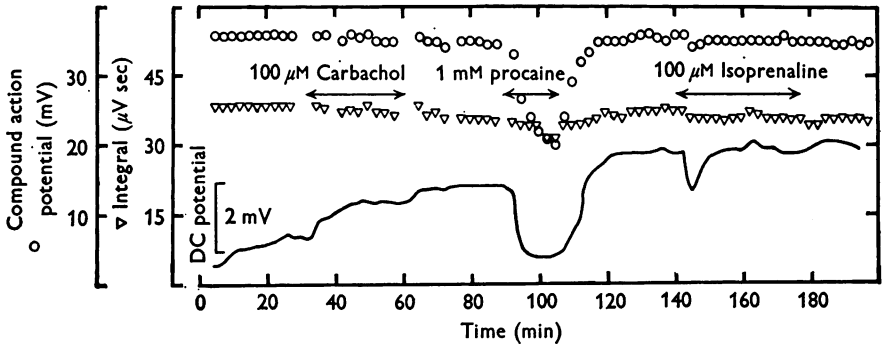

Fig. 5. The effect of neurotransmitter mimetics on the action potential amplitude (○), integral (▽), and DC potential (continuous line) in desheathed sciatic nerve as measured by the sucrose gap technique. The nerve was continuously superfused (0·2–0·3 ml./min) with normal Ringer or Ringer containing 100 μM carbachol, 1 mM procaine, or 100 μM isoprenaline (indicated by the arrows), and supramaximally stimulated once every 2·5 min. A small amount of procaine left in the reservoir tubing caused a transient depression of the signals at the start of isoprenaline superfusion. The DC potential measured across the sucrose gap is proportional to the resting membrane potential and a downward deflexion indicates depolarization.

Desheathed nerves. Since the sheath and epineurium of the sciatic nerve may limit the penetration of exogenous compounds (Feng & Liu, 1949; Crescitelli, 1951; Ritchie & Greengard, 1966), experiments were performed on desheathed nerves to increase the likelihood that the exogenous agents would penetrate to the axoplasm.

Fig. 6 illustrates typical results obtained from three different nerves. Both procaine and tetrodotoxin were more effective in blocking the conducted compound action potential in desheathed nerves than in nerves where the sheath and epineurium remained intact (Table 1). For example, the compound action potential was completely inhibited within 5 min by tetrodotoxin on desheathed nerves but only depressed 10–20% in sheathed nerves.

However, theophylline and the cyclic nucleotides did not affect the magnitude of the compound action potential in these desheathed nerves.

TABLE 1. Effect of various substances on the compound action potential of the frog sciatic nerve

Substance and concentration (mM)		Time of exposure (min)	Compound action potential amplitude (% of control ± S.E. of mean (*n*)) Sheathed	Desheathed	Compound action potential integral (% of control ± S.E. of mean (*n*)) Sheathed	Desheathed
Cyclic AMP	1·0	30	104 (1)	99 ± 1 (5)	103 (1)	101 ± 1 (5)
Monobutyryl cyclic AMP	1·0	30	102 (1)	99 ± 1 (3)	99 (1)	96 ± 3 (3)
Cyclic GMP	0·3	30	94 ± 7 (4)	—	105 ± 18 (4)	—
	1·0	30	101 (1)	102 (1)	102 (1)	101 (1)
Dibutyryl cyclic GMP	0·7	20	—	100 (1)	—	100 (1)
	1·0	30	103 (1)	98 (1)	102 (1)	103 (1)
Caffeine	5·0	30	—	97 ± 4 (2)	—	99 ± 3 (2)
RO 20-1724	0·4	30	—	91 ± 1 (2)	—	101 ± 2 (2)
Theophylline	5·0	30	103 ± 2 (6)	97 ± 3 (6)	94 ± 4 (2)	95 ± 2 (6)
Theophylline (5·0) *and* cyclic AMP *or* monobutyryl cyclic AMP	1·0	30	101 ± 3 (2)	91 ± 3 (3)	98 ± 5 (2)	93 ± 4 (3)
Carbachol	0·1	15	—	102 ± 2 (2)	—	103 ± 4 (2)
		30	—	100 (1)	—	99 (1)
Isoproterenol	0·1	30	—	100 ± 1 (3)	—	99 ± 2 (3)
Procaine	1·0	10	70 ± 2 (4)	13 ± 4 (18)	107 ± 5 (4)	33 ± 10 (18)
Tetrodotoxin	10 nM	5	81 ± 18 (2)	0 ± 0 (5)	90 ± 4 (2)	0 ± 0 (5)

Summary of results from experiments using sucrose gap or palladium electrode recording techniques. On many experiments there was a small but steady decline (~10%) over a 4 hr period in the magnitude of the compound action potential. In order to compute an unbiased % of control all drug-free periods during each experiment were considered as control periods and subjected to linear regression analysis. The resultant linear regression line for each experiment was used to predict the control value during a drug period and was compared to the observed value in order to calculate the % of control. The values above are the average ± S.E. of mean of one trial from each of (*n*) nerves. Each nerve was exposed to only one to three different drugs and a local anaesthetic. Errors due to non-linearity in decline of control values and variations in inter-electrode impedance were estimated to be ± 5%. In the table above only procaine and tetrodotoxin significantly affected the magnitude of the compound action potential.

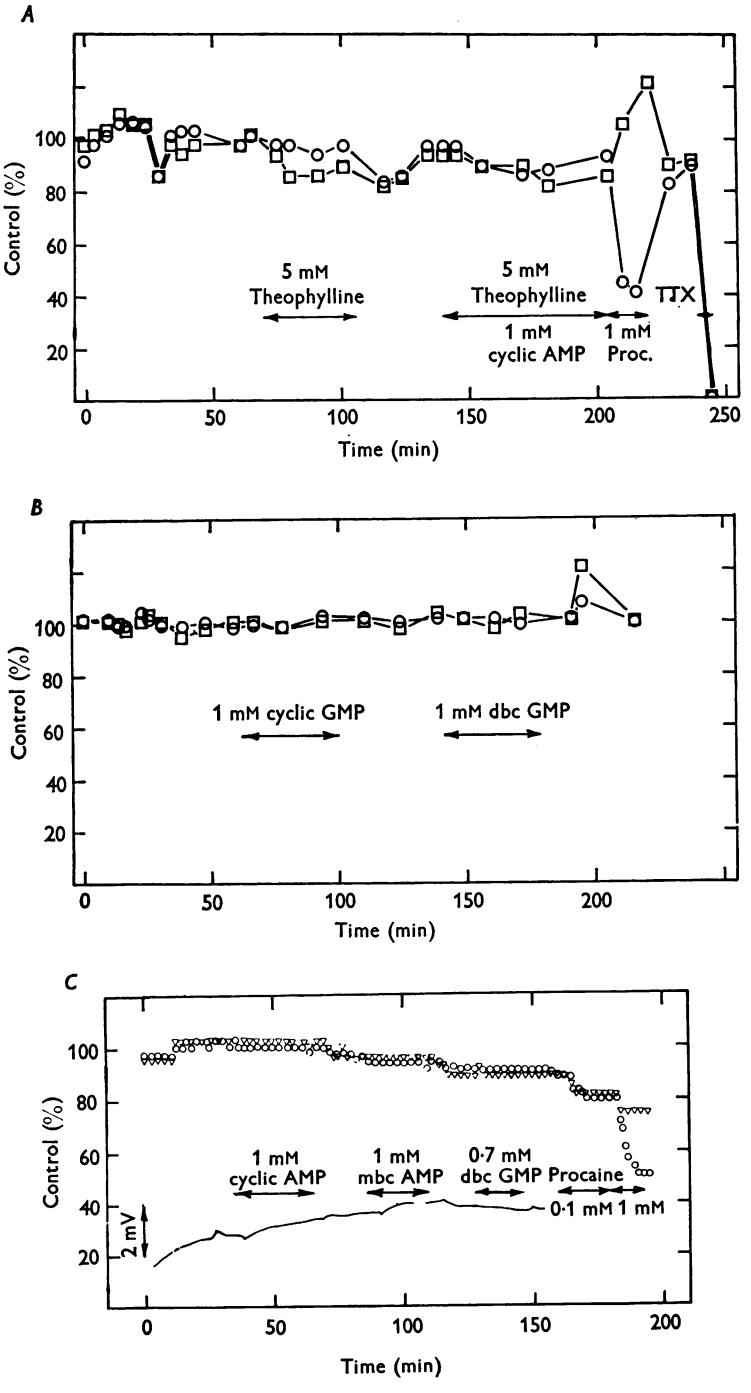

Fig. 6. For legend see facing page.

Three different phosphodiesterase inhibitors (caffeine, RO 20–1724, theophylline) and the more permeable butyryl derivatives of the cyclic nucleotides were tested at relatively high concentrations (Table 1). In addition three sucrose gap experiments demonstrated that the cyclic nucleotides did not change the resting potential of the frog sciatic nerve (Fig. 6).

DISCUSSION

The results presented above demonstrate that exogenous cyclic nucleotides have no significant effect on the magnitude of compound action potential or resting potential in frog sciatic nerve. The concentration of the cyclic nucleotides and phosphodiesterase inhibitors employed were similar to those used in other tissues, including nervous tissue, to produce a response which mimicked a cyclic nucleotide dependent hormone effect (Robison *et al.* 1971; McAfee & Greengard, 1972; Kalix, McAfee, Schorderet & Greengard, 1974). Attempts were made to increase the sensitivity of the axon to the applied cyclic nucleotide by use of the more permeable butyryl derivatives, phosphodiesterase inhibitors to reduce degradation and increase endogenous cyclic nucleotide levels, and desheathing to reduce penetration barriers. The mechanical reduction in penetration barriers was effective since local anaesthetics such as procaine and tetrodotoxin were more effective and quicker acting on desheathed nerves.

These observations in axonal tissue are in contrast to observations elsewhere in the nervous system. Exogenous cyclic nucleotides and phosphodiesterase inhibitors seem able to penetrate and affect the excitability of certain neurones presumably at presynaptic or post-synaptic sites (McAfee & Greengard, 1972; Siggins, Oliver, Hoffer & Bloom, 1971; Dretchen, Standaert, Skirboll & Morgenroth, 1976; however, see Lake & Jordan, 1974; Miyamoto & Breckenridge, 1974). Because the several

Fig. 6. The amplitude of the compound action potential (○) and its integral (□) during exposure of *desheathed* nerves to cyclic nucleotides, theophylline, and local anaesthetics. The values are plotted as % of the responses preceding the first exposure to drug-containing Ringer. Parts *A* and *B*: data obtained from compound action potential recorded via extracellular palladium electrodes. The arrows mark the onset and termination of periods of exposure to Ringer containing the experimental substances. The concentration of tetrodotoxin (TTX) in part *A* was 10 nM. Part *C*: sucrose gap recordings of compound action potential (○) and integral (▽) as % of control and DC potential across the gap (continuous line). These parameters were continuously monitored while the nerve was stimulated at 1/min and superfused (0·2–0·3 ml./min) with normal Ringer or Ringer containing cyclic nucleotides and procaine. For convenience every third data point is plotted.

methods used to increase tissue cyclic nucleotide content failed to alter the compound action potential or resting potential, myelinated frog axons, unlike synaptic tissue, probably lack the machinery by which cyclic nucleotides control membrane excitability.

We have no explanations for the disparity between our results and a short report (Vande Berg, 1974) where a 30 min exposure to cyclic AMP or phosphodiesterase inhibitors decreased by 50% the compound action potential of sheathed frog sciatic nerves. McAfee & Greengard (1972) reported that cyclic GMP slightly depolarized the rabbit vagus nerve. However, in these studies neither cyclic AMP nor cyclic GMP significantly affected the C fibre elevation of the vagus nerve during a 5 min exposure. Furthermore, theophylline (2·5 mM) was innocuous to vagal conduction of action potentials over a 30 min period. Further experimentation is necessary to determine if certain fibre types (e.g. unmyelinated axons) or axons of certain species are more sensitive to exogenous cyclic nucleotides than the frog myelinated nerve.

Of considerable interest is the observation that isoprenaline and carbachol increase the cyclic nucleotide content of sciatic nerve. Based on the nature of the agonists and antagonists used in this study we tentatively conclude that activation of β-adrenergic receptors in the nerve augments cyclic AMP and muscarinic activation augments cyclic GMP. However, agonist dose–response studies and other specific antagonists need to be employed to unambiguously identify the receptors which augment the cyclic nucleotides. The concentration of the agonists and antagonists used in this study are relatively high but comparable to those employed in superfused autonomic ganglia to maximally augment cyclic nucleotide levels (Kalix *et al.* 1974; Weight, Petzold & Greengard, 1974; Greengard & Kebabian, 1974). In fact the concentration of carbachol was less by tenfold than the dose of acetylcholine needed to produce a change in the compound action potential of the rabbit vagus nerve (Armett & Ritchie, 1960). The cell type upon which these receptors are located is unknown. However, the nerve sheath was not responsive to isoprenaline. There was no electrogenic effect of either adrenergic or cholinergic agonists as measured by the sucrose gap technique. It is possible that these agonists act on Schwann cells or related periaxonal connective tissue to augment cyclic nucleotides. It is also possible that the cyclic nucleotides increase in the axoplasm but that they control processes other than membrane excitability. For instance cyclic nucleotides have been implicated in the control of neuronal metabolism, microtubule function and axoplasmic transport (Costa, Guidotti & Hanbauer, 1974; Goodman, Rasmussen, Di Bella & Guthrow, 1970; Sloboda, Rudolph, Rosenbaum & Greengard, 1975; Boegman & Wood, 1976).

Application of exogenous neurotransmitter receptor agonists augment cyclic nucleotides in the nervous system and this result is often assumed to occur in the subsynaptic regions of neurones. Our results clearly demonstrate that these agents can augment cyclic nucleotide levels in nonsynaptic nervous tissue. Thus it will be important to exclude responses from nonsynaptic nervous tissue in any experiments purporting to demonstrate cyclic nucleotide dependent synaptic transmission.

We wish to acknowledge the excellent technical assistance of Gary Whiting in performing biochemical assays and Alina Cosio in preparation of the manuscript. This work was supported by USPHS Grants NS 11552 and NS 05820, and the Palm Beach Chapter of the Florida Heart Association.

REFERENCES

ARMETT, C. J. & RITCHIE, J. M. (1960). The action of acetylcholine on conduction in mammalian non-myelinated fibres and its prevention by an anticholinesterase. *J. Physiol.* **152**, 141–158.

BLOOM, F. E. (1975). The role of cyclic nucleotides in central synaptic function. *Rev. Physiol. Biochem. Pharmacol.* **74**, 1–103.

BOEGMAN, R. J. & WOOD, P. L. (1976). Monoamines as possible mediators in the regulation of fast axoplasmic flow. *J. Neurochem.* **26**, 737–740.

COSTA, E., GUIDOTTI, A. & HANBAUER, I. (1974). Do cyclic nucleotides promote the trans-synaptic induction of tyrosine hydroxylase? *Life Sci. Oxford* **14**, 1169–1188.

CRESCITELLI, F. (1951). Nerve sheath as a barrier to the action of certain substances. *Am. J. Physiol.* **166**, 229–240.

DRETCHEN, K. L., STANDAERT, F. G., SKIRBOLL, L. R. & MORGENROTH, V. H. (1976). Evidence for a prejunctional role of cyclic nucleotides in neuromuscular transmission. *Nature, Lond.* **264**, 79–81.

FENG, T. P. & LIU, Y. M. (1949). The connective tissue sheath of the nerve as effective diffusion barrier. *J. cell. comp. Physiol.* **34**, 1–32.

GOLDBERG, N. D., O'DEA, R. F. & HADDOX, M. K. (1973). Cyclic GMP. In *Advances in Cyclic Nucleotide Research*, vol. 3, ed. GREENGARD, P. & ROBISON, G. A. New York: Raven.

GOODMAN, D. B. P., RASMUSSEN, H., DI BELLA, F. & GUTHROW, C. F. (1970). Cyclic adenosine 3′:5′-monophosphate-stimulated phosphorylation of isolated neurotubule subunits. *Proc. natn. Acad. Sci. U.S.A.* **67**, 652–659.

GREENGARD, P. (1976). Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters. *Nature, Lond.* **260**, 101–108.

GREENGARD, P. & KEBABIAN, J. W. (1974). Role of cyclic AMP in synaptic transmission in the mammalian peripheral nervous system. *Fedn Proc.* **33**, 1059–1067.

HORN, J. P. & MCAFEE, D. A. (1976). Peripheral nerve: levels of cyclic nucleotides and their effect on excitability. *Fedn Proc.* **35**, 424.

KALIX, P., MCAFEE, D. A., SCHORDERET, M. & GREENGARD, P. (1974). Pharmacological analysis of synaptically mediated increase in cyclic adenosine monophosphate in rabbit superior cervical ganglion. *J. Pharmac. exp. Ther.* **188**, 676–687.

KEBABIAN, J. W., STEINER, A. L. & GREENGARD, P. (1975). Muscarinic cholinergic regulation of cyclic guanosine 3′, 5′-monophosphate in autonomic ganglia: possible role in synaptic transmission. *J. Pharmac. exp. Ther.* **193**, 474–488.

KOSTERLITZ, H. W., LEES, G. M. & WALLIS, D. I. (1968). Resting and action potentials recorded by the sucrose-gap method in the superior cervical ganglion of the rabbit. *J. Physiol.* **195**, 39–53.

LAKE, N. & JORDAN, L. M. (1974). Failure to confirm cyclic AMP as second messenger for norepinephrine in rat cerebellum. *Science, N.Y.* **183**, 663–664.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, A. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

MCAFEE, D. A. & GREENGARD, P. (1972). Adenosine 3′,5′-monophosphate: electrophysiological evidence for a role in synaptic transmission. *Science, N.Y.* **178**, 310–312.

MIYAMOTO, M. D. & BRECKENRIDGE, B. M. (1974). A cyclic adenosine monophosphate link in the catecholamine enhancement of transmitter release at the neuromuscular junction. *J. gen. Physiol.* **63**, 609–624.

RITCHIE, J. M. & GREENGARD, P. (1966). On the mode of action of local anaesthetics. *A. Rev. Pharmac.* **6**, 405–429.

ROBISON, G. A., BUTCHER, R. W. & SUTHERLAND, E. W. (1971). *Cyclic AMP.* New York: Academic.

SIGGINS, G. R., OLIVER, A. P., HOFFER, B. J. & BLOOM, F. E. (1971). Cyclic adenosine monophosphate and norepinephrine: effects on transmembrane properties of cerebellar purkinje cells. *Science, N.Y.*, **171**, 192–194.

SLOBODA, R. D., RUDOLPH, S. A., ROSENBAUM, J. L. & GREENGARD, P. (1975). Cyclic AMP-dependent endogenous phosphorylation of a microtubule-associated protein. *Proc. natn. Acad. Sci. U.S.A.* **72**, 177–181.

STEINER, A. L. (1973). Radioimmunoassay for the cyclic nucleotides. *Pharmac. Rev.* **25**, 309–313.

VANDE BERG, J. S. (1974). Inhibitory effects of dibutyryl and cyclic AMP on the compound action potential in the frog (*Rana pipiens*) sciatic nerve. *Experientia* **30**, 1025–1027.

WEIGHT, F. F., PETZOLD, G. & GREENGAARD, P. (1974). Guanosine 3′,5′-monophosphate in sympathetic ganglia: increase associated with synaptic transmission. *Science, N.Y.* **186**, 942–944.