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The effect of cold storage on the inhibitory action of isoprenaline, phenylephrine and nicotine on the mechanical and membranal activities of guinea-pig taenia caecum

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Summary

1. The effects of prolonged cold storage on the mechanical and membranal responses to stimulation of α- and β-adrenoceptors by phenylephrine and isoprenaline, respectively, were studied on the guinea-pig taenia caecum.

2. Cold storage invariably caused a decrease in the resting membrane potential, and this effect was enhanced as the duration of treatment was prolonged.

3. After cold storage (18 days) the tissue potassium ion content ($89{\cdot}7 \pm 1{\cdot}7$ mmol/kg wet wt.) was decreased to $30{\cdot}5 \pm 1{\cdot}9$ mmol/kg wet wt. whereas that for sodium ($69{\cdot}2 \pm 1{\cdot}4$ mmol/kg wet wt.) increased to $134{\cdot}0 \pm 2{\cdot}3$ mmol/kg wet wt.

4. In the fresh preparations, phenylephrine (1 and 2 μM) caused a cessation of spontaneous action potentials, accompanied by hyperpolarization of the membrane and relaxation of the muscle. These effects were markedly diminished after 18 days of cold storage. Isoprenaline (1 and 2 μM) also blocked the action potentials and caused a concomitant muscle relaxation, but in most cases the hyperpolarization was not observed. After 14 days of cold storage these mechanical and membranal changes associated with isoprenaline treatment were not demonstrable in most preparations.

5. Nicotine (5 μM and 50 μM) produced a biphasic effect, cessation of the action potential, hyperpolarization and subsequent relaxation followed by a long lasting depolarization, an accelerated discharge of action potentials and an increase in muscle tension. After a few days of cold storage the hyperpolarization effect disappeared but the intensity of the long-lasting depolarization as well as the contractile effects were increased. After cold storage for more than 7 days, nicotine did not affect mechanical or electrical activity.

6. Dibutyryl 3′5′ cyclic AMP (1 μM to 500 μM) failed to affect the mechanical and electrical activities of taenia caecum.

7. Phenylephrine and isoprenaline had no effect on the high potassium-depolarized taenia.

8. These observations suggest that the electro-mechanical effect of an α-adrenoceptor stimulant on the guinea-pig taenia caecum is more resistant to cold treatment than that of a β-adrenoceptor stimulant. This inhibitory system

of stimulation of both α- and β-receptors of guinea-pig taenia caecum may react by different mechanisms. The results also demonstrate that cold storage itself changes the membrane permeability to ions and the tissue ion content (Na^+ and K^+) of smooth muscle of guinea-pig taenia caecum.

Introduction

In spite of the numerous studies on the nature of the adrenergic receptors in the intestinal smooth musculature, our knowledge is at best, sketchy. Part of the problem arises from the difficulty associated with the functional separation of the different receptor types. In an analysis of the α- and β-adrenoceptor activities, Shibata, Hattori & Timmerman (1970) previously observed that cold storage of the guinea-pig taenia caecum (up to 7 days) had no effect on the relaxation produced by phenylephrine, an α-adrenoceptor stimulant and isoprenaline, a β-adrenoceptor stimulant; that is, both receptor activities remained functional. However, no studies have been made on the influence of prolonged cold storage (more than 10 days) on the mechanical and electrical responses of the intestinal smooth muscle to α- and β-adrenoceptor stimulation.

Recently, we have found that the inhibitory action of phenylephrine, but not that of isoprenaline, persists even after 18 days of cold storage. Therefore, a further study was made on the mechanical and membranal response of the taenia caecum of the guinea-pig to phenylephrine, isoprenaline and nicotine after prolonged cold storage (up to 18 days).

Methods

In this study guinea-pigs of either sex, weighing on average 500 g, were used. The animals were killed by a blow on the head, the carotids cut, and the abdominal cavity opened to dissect the taeniae caeca. The taeniae caeca obtained from the same animal were cut into several strips (each about 0·8 cm long) and half of them were used as fresh preparations and the remainder as cold storage preparations. The strips were mounted almost horizontally in an organ bath of 40 ml volume filled with Ringer solution of the following composition (mM): NaCl, 153·9 ; $CaCl_2$, 2·4 ; KCl, 5·4 ; $NaHCO_3$, 6·0 ; dextrose, 11·0 in distilled, deionized water. High potassium medium was made by replacement of the sodium chloride in Ringer solution with potassium chloride. These media were maintained at pH 7·3 and at 37° C and constantly equilibrated with a gas mixture of 95% O_2 and 5% CO_2. Ligatures were placed at both ends of a muscle strip, one end secured to a glass holding device mounted on a micromanipulator and the other end to a force transducer to record the muscle contractions isometrically. For cold storage treatment the strips were kept in the refrigerator at $2{\cdot}0 \pm 0{\cdot}5°$ C, in 100 ml Ringer solution, without an exogenous oxygen supply for one to 18 days. After treatment, the cold stored strips were transferred to warm Ringer (37° C) and then incubated for a similar three hours before the experimental procedure was initiated. A similar equilibration time was allowed for the fresh preparation. Muscle tension changes were recorded by a strain gauge transducer (Grass, FT.03) and a Grass 7 polygraph.

All agents were prepared immediately before use from concentrated stock solutions which had been refrigerated at 2° C. These solutions were made with distilled, deionized water. The concentrations of these agents are expressed as the final concentration in the tissue bath.

The following agents were used: (±)-isoprenaline sulphate, phenylephrine hydrochloride, phentolamine hydrochloride, dibutyryl 3′5′ cyclic AMP, nicotine and MJ 1999 (Sotalol, (±)-4-(2-isopropylamino-1-hydroxyethyl) methane sulphonanilide HCl).

Measurement of electrical activity

For measurement of membrane potential changes, glass microelectrodes filled with 3 M KCl with tip resistance of 50 to 100 MΩ were used. The electrodes were mounted flexibly as floating electrodes and as described by Hukuhara & Fukuda (1968). The recording electrode was connected through a microelectrode D.C. amplifier (Grass, Model P16) to an oscilloscope (Tektronix, Type 565) and polygraph. Oscilloscope monitoring showed that the actual values of the action potential amplitude were about 25% greater than those displayed by the pen-recorder. Both tension and membrane potential changes were recorded simultaneously.

Measurement of the ion content

The strips, weighing 20–40 mg, were suspended in a tissue bath for 4 h and dried for about 20 h at 95° C. Tissue water content was determined by subtracting the dry from the wet weight. The ion contents of the samples were measured by a flame photometer (Instrumentation Laboratory, Model 147), as described by Kao (1961). The value of orbital space (Casteels, 1969) was taken as intercellular space. The intracellular ion concentration was calculated per litre fibre water according to the method of Boyle, Conway, Kane & O'Reilly (1941). The equilibrium potential was calculated by the Nernst equation.

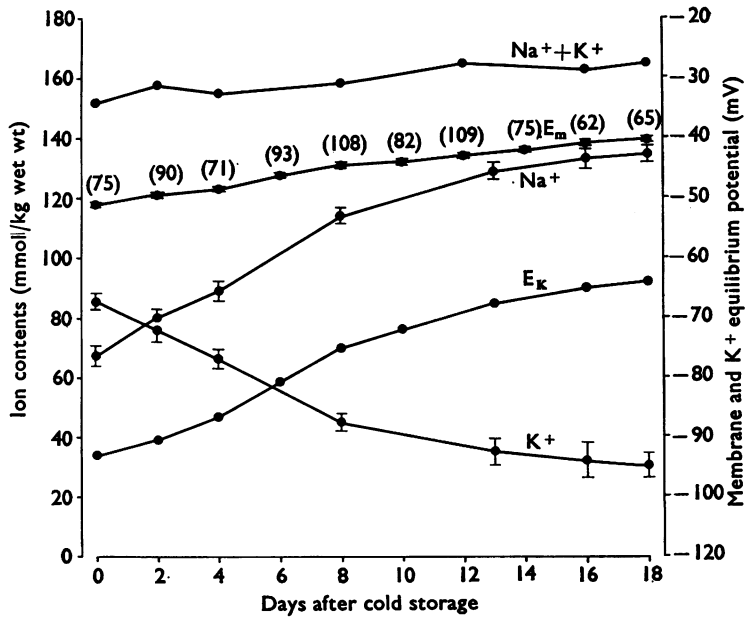

FIG. 1. The effect of prolonged cold storage on the membrane and potassium equilibrium potential (E_K) and tissue ion contents (Na^+ and K^+) of guinea-pig taeniae caeca. Vertical lines indicate the S.E.M. of the determination. The numbers in parentheses express the number of measurements of membrane potential (E_m).

Results

Electrical activity

Cold storage of taenia caecum progressively decreased the membrane potentials. In twenty separate fresh preparations, the mean membrane potential was $-51{\cdot}0 \pm 0{\cdot}3$ mV ; after 18 days of cold storage, the membrane potential for a similar number of strips was $-40{\cdot}5 \pm 0{\cdot}5$ mV (Fig. 1). Cold storage treatment noticeably affected the prepotential and the configuration of the slow wave following the action potential which was composed of an after hyperpolarization, a late after depolarization and a delayed after hyperpolarization (Fig. 2). After 2–4 days of cold storage and as a

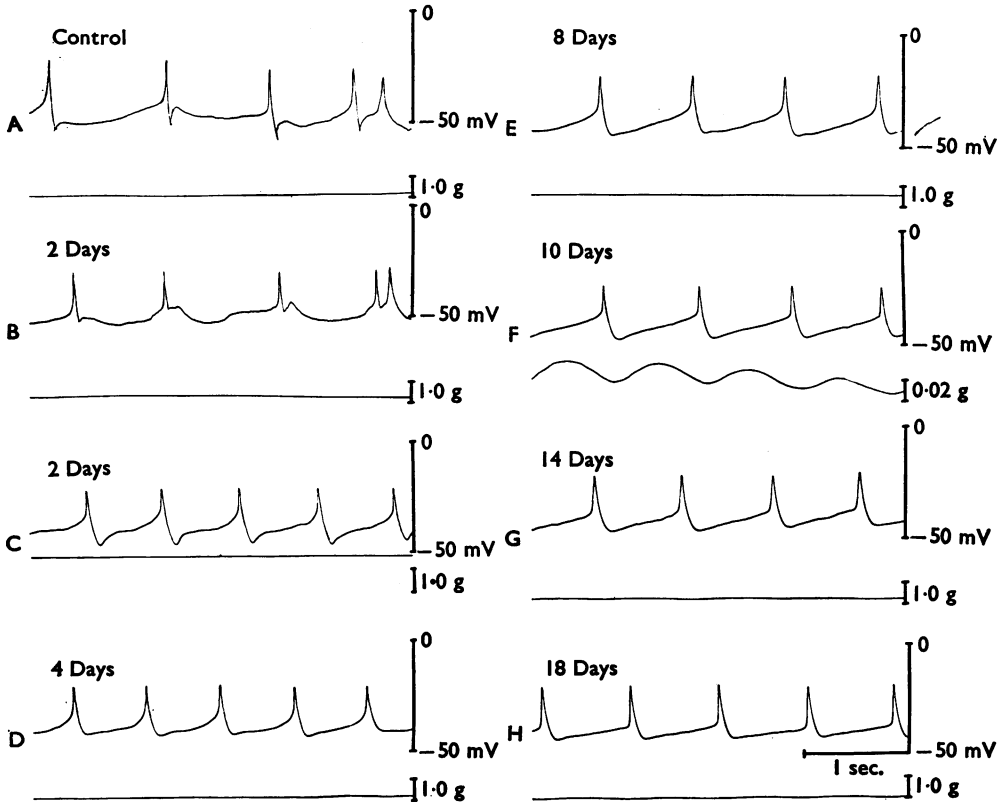

FIG. 2. The effect of cold storage on the action potential and slow wave of guinea-pig taeniae caeca. Upper and lower lines indicate the electrical and mechanical activities, respectively. Note the different tension scale (g) of F.

result of the decreased amplitude of the late after depolarization and the delayed after hyperpolarization (Fig. 2 B–D), the configuration of the slow wave resembled that of the cardiac pacemaker potential. The cold storage effects became more pronounced with more prolonged (8–18 days) cold storage (Fig. 2 E–H). However, beyond a certain period of cold storage (10–12 days), overshoot of the action potentials was not apparent.

Tissue ion contents

The membrane potential changes of taenia caecum after prolonged cold storage probably reflect changes in membrane permeability to ions (Na^+ and K^+) and consequential modification of intracellular ion content. Therefore, the effect of prolonged cold storage on the cellular ion content of taeniae caeca was measured.

The potassium ion content decreased during cold storage whereas the sodium ion content increased and this effect intensified as the duration of treatment was prolonged (Fig. 1). In fresh preparations (15 observations), the potassium and sodium ion contents were $89{\cdot}7 \pm 1{\cdot}7$ and $69{\cdot}2 \pm 1{\cdot}4$ mmol/kg wet weight, respectively, whereas after 18 days of cold storage, the potassium concentration was $30{\cdot}5 \pm 1{\cdot}9$ mmol/kg wet wt. and the sodium ion concentration, $134{\cdot}0 \pm 2{\cdot}3$ mmol/kg wet wt. (Fig. 1). These values for both ions in fresh taeniae caeca are comparable to those presented by others (Goodford & Hermansen, 1961 ; Casteels & Kuriyama, 1966 ; Casteels, 1969). With prolonged cold storage, both potassium equilibrium and membrane potentials were progressively decreased. However, since the rate of decline of the potassium equilibrium potential was much greater than that of the membrane potential, the potassium equilibrium potential value approached the membrane potential with prolonged cold storage (Fig. 1).

Phenylephrine and isoprenaline responses

In order to stimulate α- and β-adrenoceptors separately, phenylephrine was given in the presence of MJ 1999 (1 μM), a β-adrenoceptor blocking agent, and isoprenaline, in the presence of phentolamine (1 μM), an α-adrenoceptor blocker. The concentrations of phentolamine and MJ 1999 were sufficient to block the respective inhibitory responses to phenylephrine and isoprenaline but did not affect the membrane potential and intrinsic mechanical activity of taenia.

When phenylephrine (1 μM) was applied to the fresh preparations, the action potential completely ceased and the membrane underwent a subsequent hyperpolarization of 10–15 mV (Fig. 3A) ; cessation of the action potentials was accompanied by muscle relaxation (Fig. 3A). Similar mechanical and electrical changes occurred in the taenia treated with isoprenaline (1 μM) except for the hyperpolarization which was very small or absent (Fig. 4A). With both drugs, inhibition of the mechanical and electrical events of both fresh and cold stored strips persisted for approximately 2 min after onset of their action. Even after 4 days of cold storage, phenylephrine and isoprenaline still increased the after-hyperpolarization and rise time of the pacemaker type potential, as in fresh preparations; the duration of the action potential was also affected in the same manner as in fresh preparations. Phenylephrine decreased the action potential duration whereas with isoprenaline there was a prolongation.

After 10 days of cold storage, the hyperpolarization produced by phenylephrine was slightly decreased (Fig. 3D) and as the cold storage period was lengthened, the duration of the inhibitory effects of phenylephrine and isoprenaline were invariably reduced. After 14 days of cold storage treatment, isoprenaline (2 μM) failed to abolish the action potential discharge, although the frequency of the action potentials was slightly decreased (Fig. 4E) ; this inhibitory activity was occasionally lost after 10 days of cold storage. Cold storage treatment nearly blocked the muscle relaxing action of isoprenaline (Fig. 4E). In contrast, phenylephrine (1 and 2 μM)

still inhibited the mechanical and electrical activities, even after 14 and 18 days of cold storage (Fig. 3E and F), but with less intensity than that observed in fresh preparations. In the present study, as previously (Shibata, Hattori & Timmerman, 1970), there was no apparent difference in the time of onset of mechanical and electrical activities of phenylephrine and isoprenaline. In most preparations of both fresh and cold stored strips, the onset of the inhibitory action of phenylephrine and isoprenaline began 60–100 seconds after drug application.

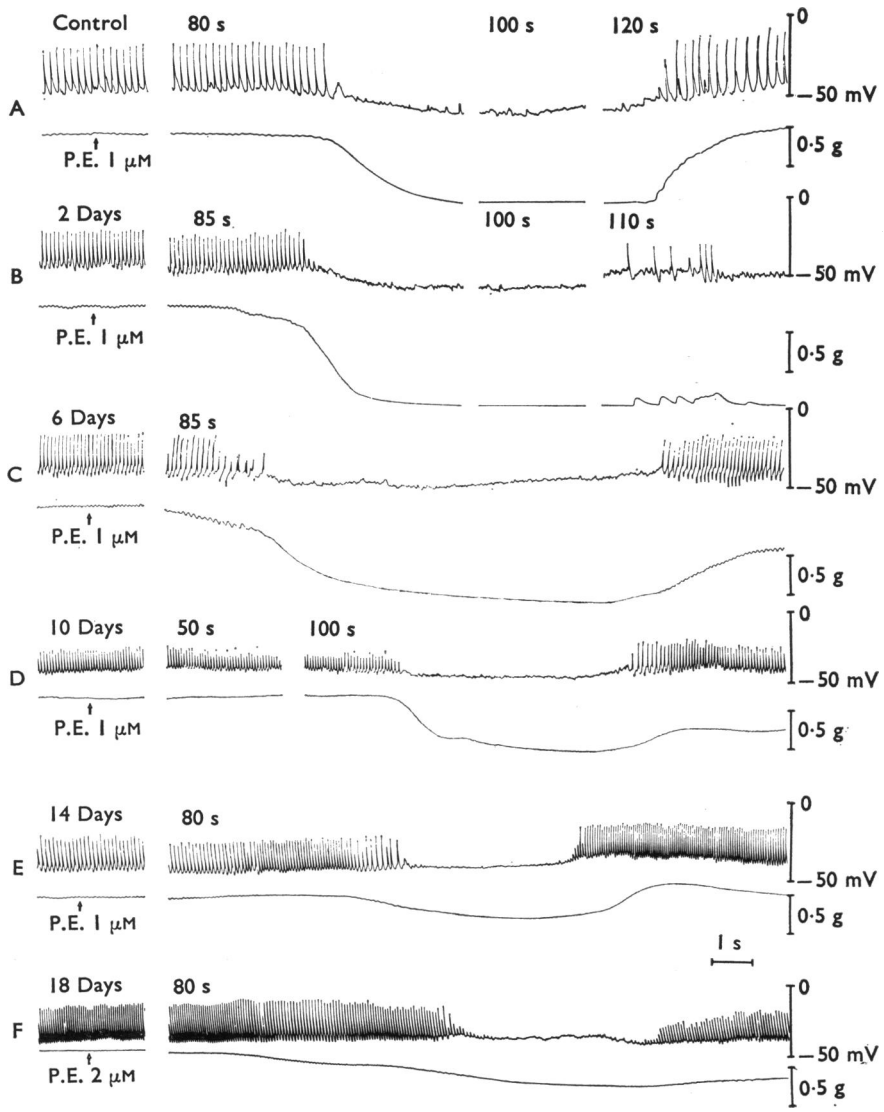

FIG. 3. The effect of cold storage on the electrical and mechanical responses of guinea-pig taeniae caeca to phenylephrine. Upper and lower lines indicate the membranal and mechanical phenomena, respectively. Arrows indicate the application of phenylephrine (PE).

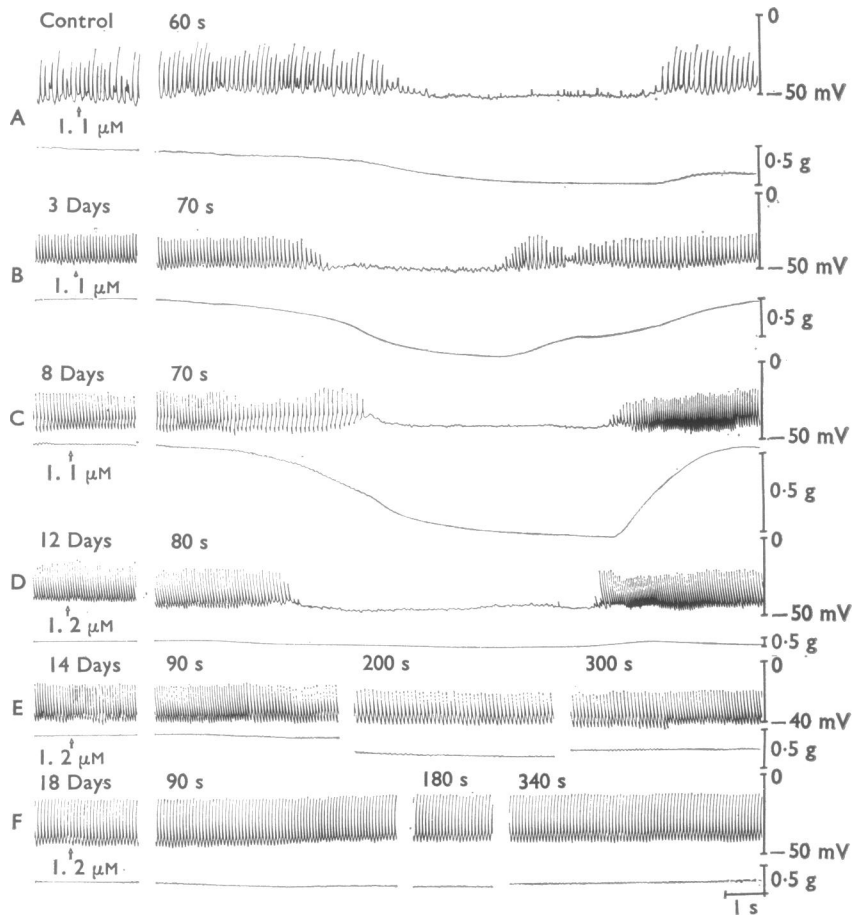

FIG. 4. The effect of cold storage on the electrical and mechanical responses of guinea-pig taeniae caeca to isoprenaline. Upper and lower lines indicate the membranal and mechanical phenomena, respectively. Arrows indicate the application of isoprenaline (I). Note the difference on the tension scale (g).

Effect of phenylephrine and isoprenaline on the depolarized muscle

In these experiments the taenia caecum was suspended vertically in the organ bath (15 ml) and the tension changes were measured by a force displacement transducer (Grass FT.03) and recorded on a Grass polygraph, as described by Shibata *et al.* (1970). Each tissue before drug treatment was initially depolarized by exposure to high potassium (153·9 mM) solution for 30 minutes.

Application of isoprenaline or phenylephrine (1 μM to 100 μM) in this preparation failed to relax the depolarized muscle. This contrasts with the results reported by Schild (1966) on uterus muscle, and by Andersson & Mohme-Lundholm (1970) on guinea-pig taenia coli, that β-adrenoceptor stimulation, but not α-adrenoceptor stimulation, relaxes potassium depolarized muscle.

Nicotine response

Figure 5 illustrates the typical electrical and mechanical effects of nicotine on fresh and cold stored taenia caecum. Three to five seconds after application of

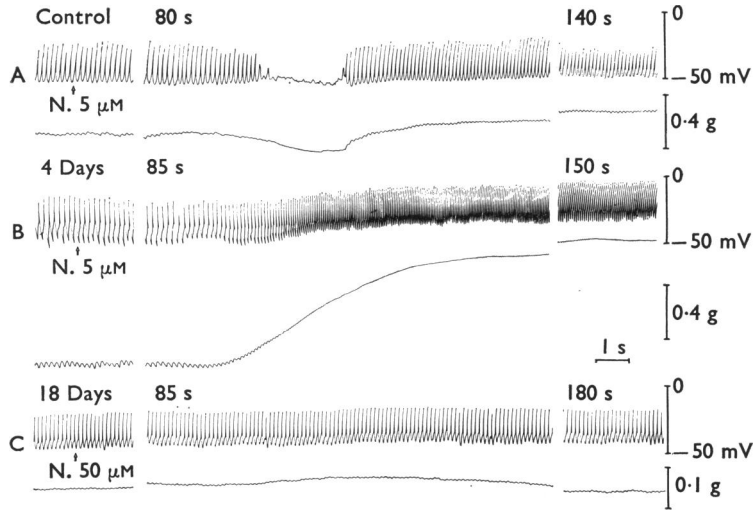

FIG. 5. The effect of cold storage on the electrical and mechanical responses of guinea-pig taeniae caeca to nicotine. Upper and lower lines indicate the membranal and mechanical phenomena. Arrows indicate the application of nicotine (N). Note the difference on the tension scale.

nicotine (5 μM), the muscle ceased to discharge action potentials, and became briefly hyperpolarized (2·5 to 5 mV). Subsequently, the muscle underwent a depolarization (4–5 mV) which led to the generation of spike potentials (Fig. 5A). The delayed contraction was associated with the subsequent regeneration of action potentials (Fig. 5A). In some cases, muscle relaxation accompanied the onset of hyperpolarization. After 4 days of cold storage, nicotine failed to relax and hyperpolarize the taenia caecum but caused a marked increase in the developed tension and in the depolarization and frequency of spike potentials (Fig. 5B). The effects of cold storage on nicotine action, the relaxation and contraction, were consistent with those previously described by Shibata, Kurahashi, Mori & Hattori (1971).

After 18 days of cold storage, the actions of nicotine (50 μM) on mechanical and electrical activities were absent (Fig. 5C).

Effect of dibutyryl 3'5' cyclic AMP (dibutyryl cyclic AMP)

Dibutyryl cyclic AMP (1 μM to 500 μM) did not affect the mechanical and electrical activities of fresh taeniae caeca. Similar results were recorded on the intestinal smooth muscle by Andersson & Mohme-Lundholm (1970) and by Levine (1968). However, 3'5' cyclic AMP relaxes fresh taeniae caeca from guinea-pig (Shibata *et al.*, 1970).

Discussion

Shibata (1969) and Shibata *et al.* (1970) have suggested that prolonged cold storage changes the permeability of membranes from rabbit aorta and guinea-pig taenia caecum to ions since such treatment interferes with the contractile response induced by high potassium concentrations. The present electrophysiological studies strongly support this suggestion.

Thus there was a direct relationship between the membrane and potassium equilibrium potentials (Fig. 6); the decreased membrane potential after prolonged cold

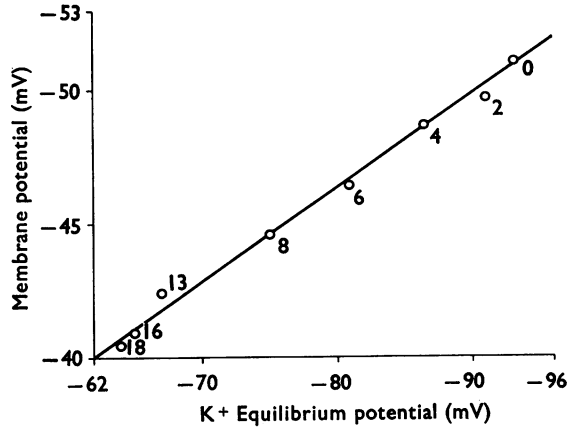

FIG. 6. The relationship between the membrane potential and potassium equilibrium potential of guinea-pig taeniae caeca. Each open circle number indicates the duration of cold storage. Zero=the fresh preparation before exposure to cold storage.

storage may reflect a decrease in the intracellular potassium concentration. However, other alternative mechanisms, such as the contributions of different ions (sodium and chloride ions), cannot be completely dismissed.

As the duration of cold storage was extended, the difference between the values of membrane and potassium equilibrium potentials gradually decreased (Fig. 1). There are several possible explanations which might explain this: (1) an increase in potassium permeability, (2) a decrease in sodium permeability and (3) an acceleration of electrogenic sodium pump activity. Mullins & Awad (1965) suggested that the elevation of intracellular sodium concentration (at low temperature) in sodium-loaded frog skeletal muscle was due to increased permeability of the muscle cell membrane to sodium. If a similar response occurs in intestinal smooth muscle after cold storage, it seems unlikely that the second possibility, that is decreased sodium permeability, would have a major effect on the ratio of membrane and potassium equilibrium potentials. Since cold storage increases the ratio of intracellular sodium to potassium, the third possibility, that is the sodium pump theory which is operative on energy supply, also becomes untenable. Therefore, the first mechanism, relating to an increased membrane permeability to potassium, seems more probable. Further study is necessary to discover the possible role of the chloride ion. Kuriyama, Osa & Toida (1967) and Hukuhara & Fukuda (1968) suggested that the late after depolarization may be mediated by a cholinergic mechanism. The disappearance of the late after depolarization by cold storage (more than 8 days) may be explained by the decrease or absence of acetylcholine release as a result of the deterioration of cholinergic nerve elements (see review of Kosterlitz & Lees, 1964).

In the intestine, the inhibitory actions of catecholamines are mediated by activation of both α- and β-adrenoceptors (Ahlquist & Levy, 1959; Levy, 1959; Furchgott, 1960). Previous results from experiments with guinea-pig taenia caecum indicated that, even after seven days of cold storage at 2° C, the relaxation produced by both α- and β-adrenoceptor stimulants was not modified (Shibata *et al.*, 1970). In the present experiments, prolonged cold storage (18 days) almost blocked completely the mechanical and electrical responses to isoprenaline, whereas the in-

hibitory action of phenylephrine persisted suggesting that β-adrenoceptors are more sensitive to cold storage than α-adrenoceptors. It is possible that the increased membrane permeability to potassium and the reduced ionic gradient across the cell membrane produced by cold storage may have blocked the inhibitory action of β- but not α-adrenoceptors. This hypothesis is not consistent with that presented by other investigators (Jenkinson & Morton, 1965, 1967; Bueding & Bülbring, 1964; Brody & Diamond, 1967), namely that β-adrenoceptor action on the intestinal smooth muscle is not directly related to alteration in potassium permeability and subsequent membrane potential change but is mediated by an intracellular metabolic action. The relaxation mediated by α-adrenoceptor stimulation is dependent upon a change in the ionic permeability of the cell membrane. Indeed, the fact that activation of α-adrenoceptors by phenylephrine caused hyperpolarization of the cell membrane of taenia caecum favours the hypothesis that the α-adrenoceptor effect involves a membrane permeability change. However, in the potassium depolarized taenia in which the ion gradient across the cell membrane is decreased, neither phenylephrine nor isoprenaline exerted any inhibitory effect on the mechanical response. The disappearance of the β-adrenoceptor effect on cold storage cannot be explained solely by decreased cell metabolism and it would seem to be partially associated with a membrane permeability change. The question, however, cannot be answered quantitatively from our results.

Several investigators have suggested that 3'5' cyclic AMP may mediate the action of catecholamines in intestinal smooth muscle (Bueding, Butcher, Hawkins, Times & Sutherland, 1966; Jenkinson & Morton, 1967). The metabolic and relaxing effects of isoprenaline can be completely stimulated by cyclic AMP (Andersson & Mohme-Lundholm, 1970).

In the present experiments, the failure of dibutyryl cyclic AMP to cause any mechanical and/or electrical changes in fresh taenia suggests that exogenous dibutyryl cyclic AMP may not be acting by the same mechanisms as endogenous cyclic AMP.

The effect of nicotine appears to be mediated through catecholamine release and was similar to that observed by the stimulation of α-adrenoceptors with phenylephrine. Previous findings have shown that the inhibitory action of nicotine on the mechanical activity of taenia is blocked equally by either α- or β-adrenoceptor blocking agents (Shibata *et al.*, 1970, 1971). The possibility exists that endogenous catecholamines released by nicotine probably activate both α- and β-adrenoceptors. Disappearance of the inhibitory action of nicotine on the taenia after several days of cold storage may be attributed to a reduction or lack of releasable endogenous catecholamine resulting from adrenergic nerve degeneration (Shibata *et al.*, 1971). Under such circumstances the contraction and depolarization induced by nicotine are now potentiated. The disappearance of nicotine-induced contraction and depolarization by a more prolonged cold storage (more than 8 days) is explicable by the decrease or absence of acetylcholine release due to deterioration of the cholinergic system.

The excitatory action of nicotine on the taenia can be blocked by atropine or hemicholinium (Shibata *et al.*, 1971). The results also suggest that the inhibitory actions elicited by stimulation of α- and β-adrenoceptors in the intestinal smooth muscle may be mediated by different mechanisms. It would seem of great im-

portance to take this into consideration in further studies concerning the mechanism by which the taenia caecum after cold storage still shows its intrinsic mechanical and electrical activities and responds to alpha-adrenoceptor stimulation but not to beta-adrenoceptor stimulation.

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