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THE EFFECTS OF PIPERAZINE ON RAT SYMPATHETIC NEURONES

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1 The neuronal effects of the anthelmintic piperazine (Pip) on rat sympathetic ganglia were studied *in vitro* by means of intracellular and extracellular recording techniques.

2 Surface potential recordings indicated that Pip (0.1–10 mM as citrate, 1–30 mM as hexahydrate) produced a sustained depolarization (reversible on washing) of rat ganglia. γ-Aminobutyric acid (GABA, 1–100 μM) also evoked reversible depolarizations but, unlike Pip, responses to the higher doses of GABA declined during a 2 min exposure. Depolarizations produced by Pip or carbachol (but not GABA) were markedly depressed by hexamethonium but only slightly by bicuculline or picrotoxin.

3 Intracellular recordings revealed that Pip-induced depolarizations were accompanied by an increase in membrane conductance and a broadening and depression of the directly-evoked spike.

4 In the presence of hexamethonium (1 mM), the responses to Pip hexahydrate and to cholinoceptor agonists were abolished, but Pip citrate still changed the spike configuration and induced membrane hyperpolarization with a small conductance increase. These residual effects were mimicked by superfusing with Na citrate or Ca^{2+}-free medium, suggesting that significant Ca^{2+} binding by the citrate anion of the Pip salt was probably responsible for the observed activity of Pip citrate in the presence of hexamethonium.

5 It is concluded that on rat ganglia Pip is a nicotinic agonist, with no detectable GABA-mimetic activity.

Introduction

Piperazine (Pip) is used extensively as an anthelmintic agent for *Ascaris* (roundworm) and *Enterobius* (pinworm) infestations. The drug causes a reversible flaccid paralysis of the nematodes; the worms can then be expelled by host intestinal peristalsis (Desowitz, 1971). Although Pip has been employed clinically for almost three decades, its exact mode of action is still unknown. The two main hypotheses advanced to explain the action of Pip on nematodes are: (i) a curariform block of cholinoceptors (Norton & De Beer, 1957; Sheth, 1975, although see Nistri & Arenson, 1978) and (ii) an agonist activity on inhibitory γ-aminobutyric acid (GABA) receptors present on *Ascaris* muscle (Del Castillo, De Mello & Morales, 1964). Interestingly, Pip also mimics the action of GABA on muscle fibres of the crayfish (Iravani, 1965) and lobster (Constanti & Nistri, 1976).

Although Pip is generally regarded as an anthelmintic with minimal untoward effects, there have been several reports of some neurotoxic effects attributable to its use (Parsons, 1971; Most, 1972; Berger, Globus & Melamed, 1979), particularly in young patients and in those with renal insufficiency or with a history of epilepsy (Miller & Carpenter, 1967). Unwanted effects include cerebellar ataxia, muscle weakness, disturbed consciousness and EEG changes (Schuch, Stephan & Jacobi, 1966; Belloni & Rizzoni, 1967; Gupta, 1976; Bomb & Bedi, 1976); Pip would thus seem capable of interfering with mammalian central and peripheral neurotransmission although the mechanism of these actions is not fully understood. The widespread use of Pip as an anthelmintic and its described neurotoxicity suggest a need for more detailed information on the effects of this drug on mammalian neurones, particularly those with a variety of receptors. A convenient model for this is the rat superior cervical ganglion *in vitro*, since its neurones respond to nicotinic and muscarinic cholinoceptor agonists (cf. Brown, 1980 for review) as well as to GABA (Adams & Brown, 1975).

Methods

Superior cervical ganglia (with suitable lengths of pre- and post-ganglionic nerve trunks attached) were

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excised from male Wistar rats (about 200 g body weight) anaesthetized with urethane (1.5 g/kg i.p.). Connective tissue sheaths were removed by microdissection in Krebs solution (previously bubbled with 95% O_2: 5% CO_2). Ganglia removed for intracellular recording were used promptly. Those intended for surface potential recording were desheathed and stored overnight in Krebs solution at 4°C; this treatment improved the reproducibility of the response by reducing the demarcation potential recorded at rest.

Surface potential recording

Changes in extracellular surface potential were measured by the 'air-gap' superfusion method of Brown & Marsh (1975). Ganglia were suspended vertically and superfused continuously (1 ml/min) with pre-warmed oxygenated Krebs solution (final temperature, 24–26°C). D.c. voltage changes were measured between two Ag/AgCl-agar electrodes placed on the postganglionic trunk and ganglion body respectively. The electrodes were connected via a d.c. calibrator to a Bryans potentiometric chart recorder. All drugs were dissolved in Krebs solution and applied by superfusion.

Intracellular recording

Intracellular recordings were obtained from single superficial neurones by means of glass microelectrodes filled with 4 M potassium acetate (40–60 MΩ). The design of the recording chamber and method of recording were recently described in detail (Brown & Constanti, 1980). Briefly, desheathed ganglia were suspended between two taut nylon nets in a Perspex chamber and bathed continuously with Krebs solution (29–30°C; bubbled with 95% O_2: 5% CO_2). Drug solutions were stored in separate reservoirs and applied via the bathing fluid. Membrane potential was recorded by a high input-impedance probe with current injection and electrode impedance/capacitance neutralization capabilities. Voltage responses were monitored on a storage oscilloscope and a potentiometric chart recorder and stored for analysis on magnetic tape. Orthodromic stimulation of neurones was via a pair of Pt electrodes placed on the preganglionic trunk.

Solutions and drugs

The composition of the Krebs solution was (mM, Analar grade): NaCl 118, KCl 4.8, $CaCl_2$ 2.52, $NaHCO_3$ 25, KH_2PO_4 1.18, $MgSO_4.7H_2O$ 1.19 and D-glucose 11 (bubbled with 95% O_2: 5% CO_2, pH 7.4). The following compounds were used: tri-piperazine di-citrate, mono-piperazine hexahydrate, hexamethonium bromide, bicuculline (base) and picrotoxin (all obtained from Sigma); carbachol hydrochloride, tetramethylammonium bromide (TMA), GABA and tri-sodium citrate (all from BDH). Piperidine hydrochloride was obtained from Aldrich Chemicals. Drug solutions were prepared in oxygenated Krebs and the pH adjusted to 7.4 with HCl or NaOH when necessary. Bicuculline hydrochloride solution was prepared immediately before use by dissolving the base in a few drops of 1 N HCl then diluting to the required concentration with Krebs (final pH 7.2). In surface potential experiments using carbachol, all solutions contained 1 μM hyoscine hydrobromide to prevent the slow muscarinic depolarization (Brown, Fatherazi, Garthwaite & White, 1980). The presence of hyoscine had no discernible effect on responses to piperazine or GABA.

Results

Surface potential responses

Superfusion of ganglia with Pip citrate (0.1–10 mM) or hexahydrate (1–30 mM) for 2 min produced reversible dose-related depolarizations (up to 1 mV and lasting up to 5 min). On a molar basis, the tri-Pip citrate salt was about three times more potent than the mono-Pip hexahydrate as expected. Both salts evoked depolarizations of similar time course and with little 'fading' during prolonged administration.

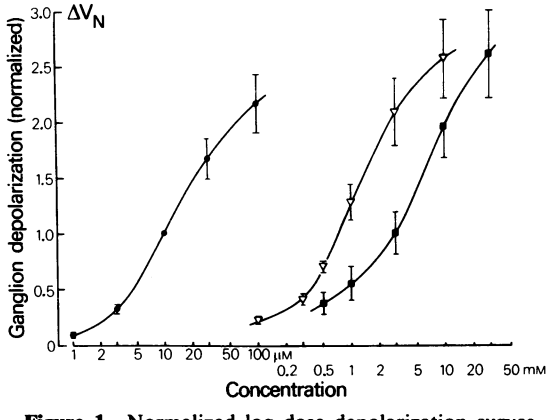

Figure 1 Normalized log dose-depolarization curves for GABA (●) and for piperazine citrate (▽) and hexahydrate (■). Ordinate scale represents ganglion depolarization measured with surface electrodes; all responses were normalized with respect to the 10 μM GABA response and plotted as the increase in normalized depolarization (ΔV_N). Abscissa scale gives drug concentration (log scale). Points are means from 9 ganglia; vertical lines show s.e.mean. Curves in this and subsequent figures were fitted by eye.

Application of Na citrate (up to 10 mM) produced only a small depolarization (< 0.2 mV), often preceded by a small hyperpolarization.

Superfusion with GABA (1–100 μM for 2 min) also depolarized, reversibly, the ganglia as previously shown (Bowery & Brown, 1974); a near maximal depolarization of about 1 mV was obtained at 100 μM. However, unlike the responses to Pip, the GABA response declined rapidly during the exposure, suggesting receptor desensitization (Adams & Brown, 1975).

Figure 1 compares pooled normalized dose-depolarization curves for GABA and Pip (as citrate or hexahydrate). In each experiment, all responses were expressed as fractions of the response to 10 μM GABA (approximate mid-point of dose-response curve). When plotted in this manner, the Pip curves had a shape apparently similar to that of the GABA curve. However, in view of the prominent fading of GABA responses at high concentrations, it was impossible to define accurately the position of the GABA maximal response. In comparison to Pip hexahydrate, GABA was approximately 300 times more potent in evoking a depolarization.

Previous work linking Pip with cholinoceptors (cf. introduction) raised the possibility of an effect of this agent on ganglionic nicotinic receptors. Pretreatment of ganglia with 1 mM hexamethonium (C_6; for 15 min) had little effect on GABA responses, but the depolarizations to Pip or carbachol were greatly depressed (Figure 2a). C_6 itself had no effect on the potential baselines. For comparable depolarizations evoked by Pip or carbachol, those induced by the latter were more effectively depressed by C_6. On closer inspection, the Pip curve in the presence of C_6 appeared flattened while the carbachol curve was displaced, in a seemingly parallel-fashion, to the right. This finding need not imply different receptor mechanisms for the two nicotinic agonists since, as shown by Rang (1966), a competitive antagonist with a slow rate of receptor dissociation may produce either parallel or non-parallel shifts of agonist log dose-response curves, depending on the degree of receptor occupancy obtained by the chosen agonist. It is possible that in our experiments with C_6, carbachol was acting as an agonist with low receptor occupancy (hence the parallel shift of the curve in Figure 2a(ii)) whereas Pip was behaving as an agonist requiring higher receptor occupancy and thus undergoing a flattening of its dose-response curve (cf. Figure 2a(iii)). It was estimated that Pip (hexahydrate) had 1/300 the agonist potency of carbachol on the ganglion.

The structural analogue of Pip, piperidine is also known to be a nicotinic agonist on autonomic ganglia (Kasé, Miyata & Yuizono, 1967). Piperidine was tested on 4 ganglia in which it produced a dose-dependent depolarization which was reversibly antagonized by 1 mM C_6. On the same preparations, the depolarizing potency of piperidine was 300 times higher than that of Pip citrate, although only 1/3 that of carbachol.

When equieffective doses of GABA or Pip were tested during a maintained depolarization to carbachol (50 μM), GABA induced a rapid repolarization of the ganglion (cf. Figure 2 of Bowery & Brown, 1974), while Pip produced either no response or a further depolarization. This suggests that the reversal potential for the actions of GABA and Pip was different, and that different ionic species might mediate the two responses. Indeed, it is known that the depolarizing action of GABA on the rat ganglion involves an outward flux of Cl ions (Adams & Brown, 1975). Thus, comparable responses to GABA, Pip and carbachol were tested in a 10 mM Cl^- medium (NaCl replaced with Na isethionate). The responses to GABA were consistently depressed, whereas responses to carbachol were usually enhanced (by 20% on average; 5 ganglia) and those to Pip either unaffected (2 ganglia; Figure 2b) or slightly increased (10% in 3 ganglia). Potentiation of nicotinic responses by low Cl^- media has also been found at the skeletal neuromuscular junction (Jenkinson & Terrar, 1973).

Further evidence that Pip was not acting via the ganglionic GABA receptor/ionophore system was provided by experiments using the two GABA antagonists, bicuculline and picrotoxin (Bowery & Brown, 1974). Bicuculline (10 μM) induced a notable shift to the right of the GABA dose-response curve but only a minimal depression of the carbachol and Pip curves (Figure 3a; see also Figure 7 of Bowery & Brown, 1974). A preferential antagonism of responses to GABA rather than to Pip or carbachol was also obtained with picrotoxin (10–100 μM) (Figure 3b).

Intracellular recordings

Results were obtained from 14 neurones (in 7 ganglia) with stable resting membrane potentials > -50 mV, spike amplitudes > 50 mV and stable input conductance (30 ± 7 nS; mean ± s.e.mean) during at least 1 h of impalement.

Bath application of Pip invariably evoked a slow depolarization of the cell always accompanied by an increase in membrane conductance. Maximal effects were observed with Pip citrate (5 mM) or Pip hexahydrate (15 mM) and consisted of a peak depolarization of up to 10 mV, associated with an average 50% rise in input conductance and a concomitant depression (or full block) of orthodromic transmission. Thresholds for the response to Pip were about 1 mM for citrate and 3 mM for hexahydrate. Depolarizing

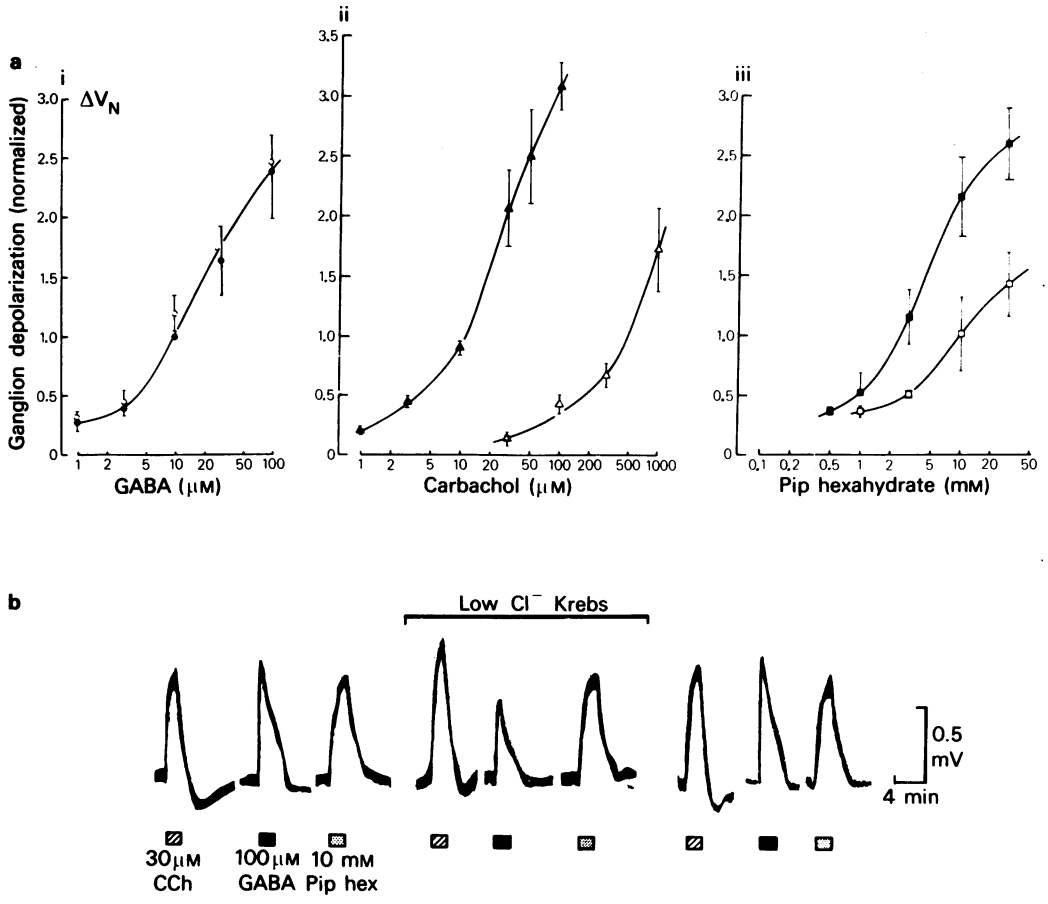

Figure 2 (a) Normalized dose-depolarization curves for GABA (i), carbachol (ii) and piperazine (Pip) hexahydrate (iii) in the presence (open symbols) or absence (filled symbols) of 1 mM hexamethonium. Ordinates: normalized surface depolarization (ΔV_N); abscissae: drug concentrations. Points are mean from 3 experiments; vertical lines show s.e.mean. Note lateral shift and depression of carbachol and Pip curves respectively; the depolarization produced by GABA was unaffected by hexamethonium. (b) Surface depolarizations recorded from a ganglion (depolarization upwards) in response to carbachol (CCh, hatched bar), GABA (filled bar) or Pip hexahydrate (Pip hex, stippled bar) (2 min applications) in normal Krebs and 20 min after changing to a low (10 mM) Cl^- medium. Note depression of GABA response. Recovery responses were obtained 15 min after return to normal Krebs. Interval between responses was 10 min.

responses to the two Pip salts are shown in Figure 4A (the effect of 1 mM piperidine on this cell is also included for comparison). In this example the hexahydrate salt was somewhat more potent than the citrate. This difference in depolarizing potency (also noted in other intracellular experiments) was not associated with any significant difference in the magnitude of the corresponding conductance increases: in fact, in systematic tests on 7 neurones in which Pip hexahydrate (15 mM) and Pip citrate (5 mM) were examined repeatedly, the conductance increase induced by the former (50% ± 10%, mean ± s.e.mean) was similar to that evoked by the latter (43 ± 9%). Figure 4B depicts the actions of the two Pip salts on the amplitude of the electrotonic potentials and on the spikes evoked by intracellular current pulses. The increase in membrane conductance induced by both Pip salts was clearly observed as a reduction in the amplitude of the hyperpolarizing electrotonic potentials; there was also broadening and depression of the direct spike (Figure 4B(iii)). Piperidine induced similar, though more rapid, effects on conductance and spike configuration (not illustrated). These changes were not replicated by passing equivalent steady depolarizing currents through the microelectrode provided that the imposed depolarization did not exceed 5 mV. Within ± 5 mV of the resting potential the current/voltage relation was linear and there-

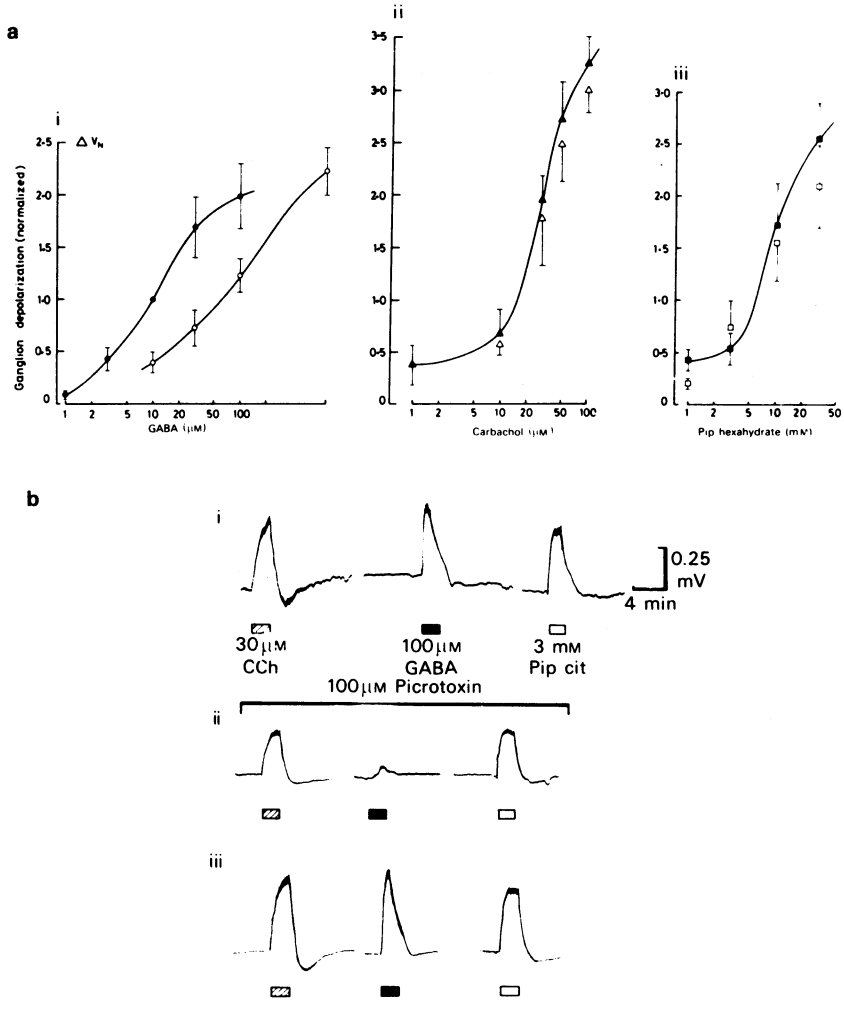

Figure 3 (a) Normalized dose-depolarization curves for GABA (i), carbachol (ii) and piperazine (Pip) hexahydrate (iii) in the presence (open symbols) or absence (filled symbols) of bicuculline (10 μM). Points are mean values ($n = 3$); vertical lines show s.e.mean. Compare the lateral shift of the GABA curve with the negligible depression of carbachol and Pip curves. (b) Effect of picrotoxin 100 μM on ganglion surface depolarization to carbachol (CCh, hatched bar), GABA (filled bar) and piperazine citrate (Pip cit, open bar). (i) Control responses; (ii) in picrotoxin (30 min pretreatment); (iii) recoveries after 40 min wash. Response intervals: 10 min.

fore the measured conductance values within these limits were not distorted seriously by rectification.

Overall, the data confirm that, when their relative Pip content is taken into account, the two Pip salts are indeed equipotent. The small difference in depolarizing activity is therefore best ascribed to an additional action of citrate ions on the neuronal membrane which limits the amplitude of the recorded depolarization. On close inspection, it became apparent that the effects of the citrate and the hexahydrate salts on the spike latency were different. With the hexahydrate salt (or nicotinic agonists such as TMA and piperidine) the spike latency decreased transiently and then increased (often leading to complete block), whereas with the citrate the decrease in latency was maintained throughout the application (at 5 mM, Pip citrate rarely produced a complete shunting of the spike).

In Figure 5 the effects of Pip citrate and GABA were compared on the same neurone. The depolarization, the accompanying conductance increase, and the depression of the direct spike (the latter not shown) produced by GABA declined rapidly during a continued application (Figure 5b(i)). A similar

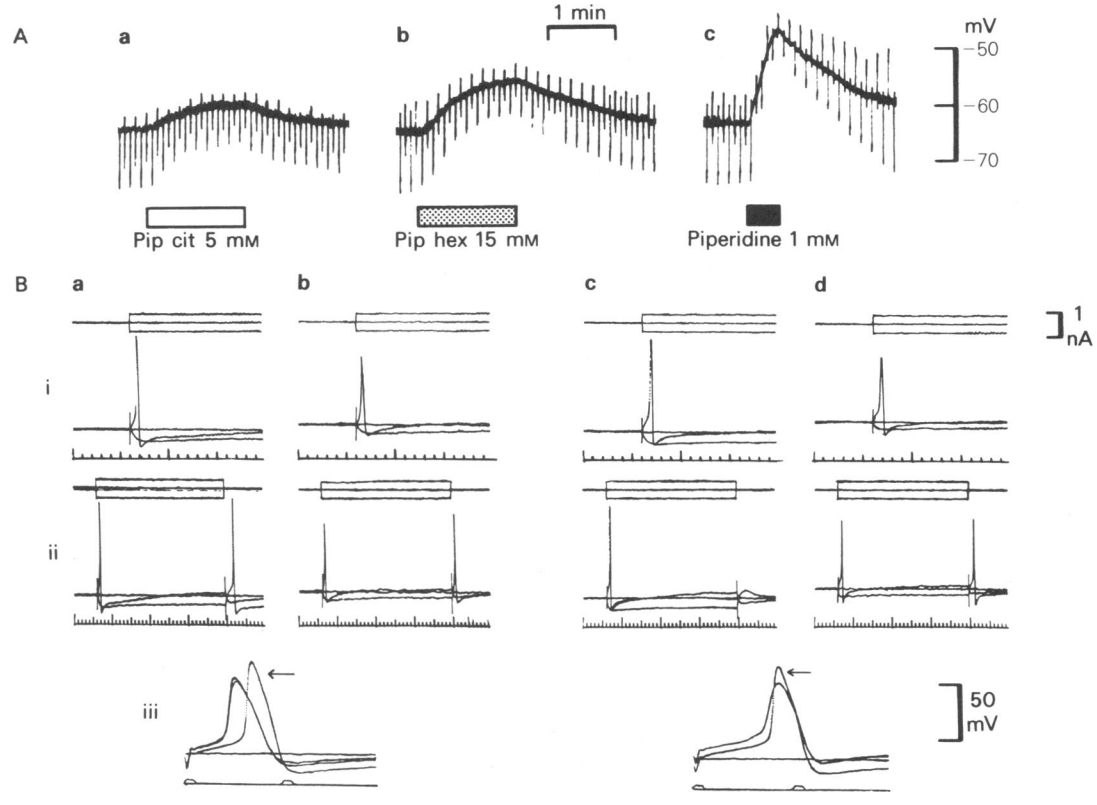

Figure 4 (A) Chart record of membrane potential of a single ganglionic neurone. Large downward deflections are hyperpolarizing electrotonic potentials (evoked by constant, long current pulses; 300 ms; − 0.4 nA); upward blips are truncated spikes produced by depolarizing current pulses (small downward deflections are spike afterhyperpolarizations). (a–c), successive depolarizing responses to piperazine (Pip) citrate, hexahydrate and piperidine applied to the same neurone during the periods indicated by the bars. (B) Corresponding oscilloscope records of responses to depolarizing and hyperpolarizing current pulses (300 ms) at fast (i) and slow (ii) sweep speeds. (Upper beam = current; middle beam = voltage; lower beam = 10 ms time marks). (a) Control; (b) at peak of Pip citrate response; (c) control; (d) at peak of Pip hexahydrate response. (iii) direct spike (evoked by + 0.4 nA) at expanded sweep. Arrows indicate control spikes. Superimposed spikes were taken at peak of responses to Pip citrate (left) or Pip hexahydrate (right).

response decline was not seen during Pip application (Figure 5b(ii). Fading of voltage responses to GABA, but not to Pip, was previously noted with extracellular recordings. In the presence of bicuculline (50 μM) the GABA response was abolished, whereas that to Pip was unaffected (Figure 5b(iii), (iv)). Application of bicuculline plus 1 mM C_6 converted the Pip citrate depolarization to a slow, waning hyperpolarization with a very small conductance increase. During this response, the latency and amplitude of the direct spike underwent changes similar to those seen previously without bicuculline or C_6 (Figure 5a). In contrast, Pip hexahydrate (not illustrated) had no significant effect on the membrane potential, conductance or direct spike of single intracellularly-recorded neurones in the presence of bicuculline (50 μM) plus C_6 (1 mM). It may be noted that the effect of the same dose of hexahydrate was simply halved by 1 mM C_6 in extracellular experiments (cf. Figure 2a(iii)) where the recorded responses reflected the average depolarization of a very large number of neurones.

Companion experiments revealed that C_6 (1 mM) was adequate to block cholinoceptors (activated by TMA or carbachol in hyoscine solution) as well as the apparent nicotinic action of Pip (Figure 6). This concentration of C_6 was also sufficient to block orthodromic ganglionic transmission.

The bicuculline- and C_6-insensitive effects of Pip citrate on the membrane potential and spike suggested that a component of action of this salt on the ganglion could have been caused by the known Ca^{2+} chelating property of the trivalent citrate anion.

Figure 7 compares the effects of Na citrate

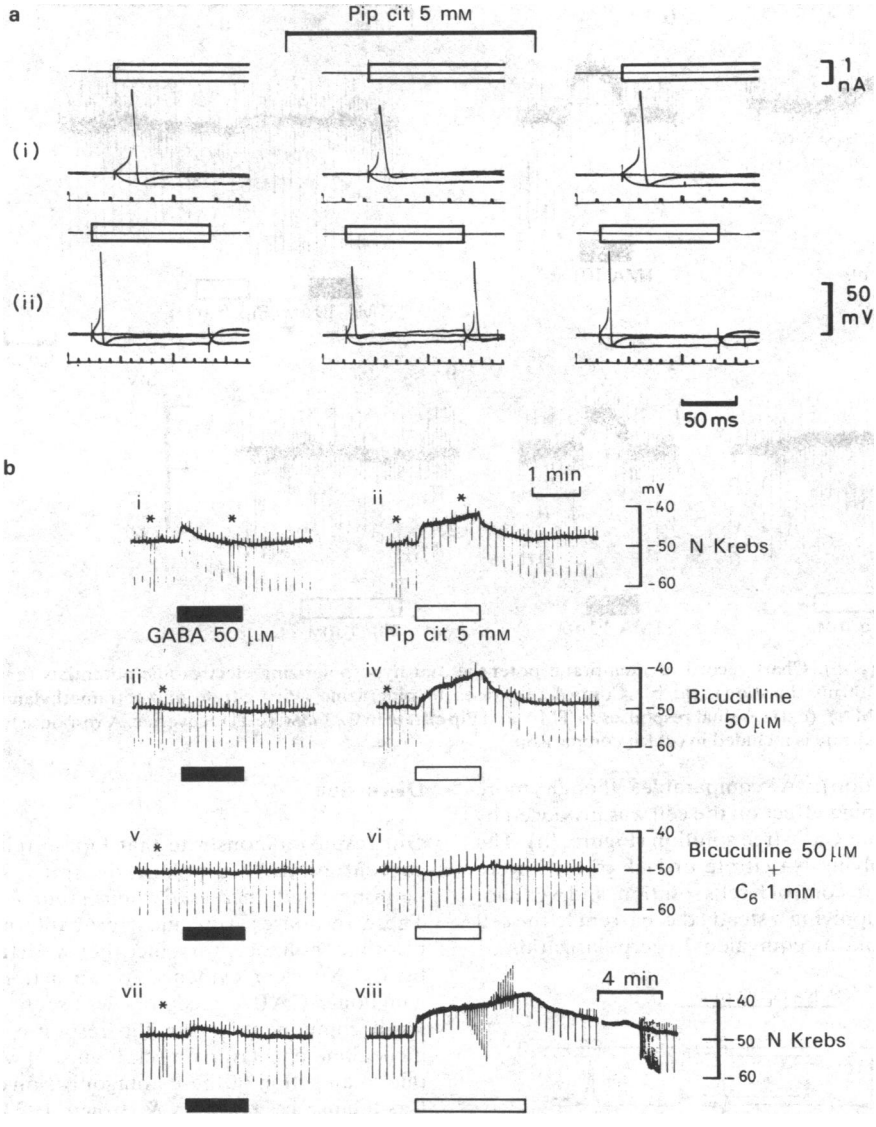

Figure 5 (a) Responses to alternate depolarizing and hyperpolarizing current pulses (300 ms; ± 0.4 nA) at two sweep speeds (fast (i) and slow (ii)) before (left), in the presence of 5 mM piperazine (Pip) citrate (middle; all in bicuculline 50 μM plus hexamethonium (C_6) 1 mM solution; these tracings correspond to response (vi) in (b)) and after washout (right) of Pip. Note depression and broadening of spike, the decrease in latency and in afterhyperpolarization, and the appearance of anode break spike. (b) (i–viii). Corresponding membrane potential record (downward deflections are hyperpolarizing electrotonic potentials due to − 0.4 nA). (i, ii): control responses to GABA and Pip citrate; note 'waning' depolarization and conductance increase to GABA; (iii, iv): in bicuculline 50 μM; (v, vi): in bicuculline + C_6 1 mM, note residual effect of Pip citrate; (vii, viii): recoveries after return to normal (N) Krebs. * = orthodromic transmission test. Large downward deflections are afterhyperpolarizations following the orthodromic spike. Note the longer application time in (viii).

(10 mM) and Ca^{2+}-free Krebs solution on the same cell. Application of citrate produced a small, waning hyperpolarization (< 4 mV) with a slight increase in input conductance; the direct spike amplitude was depressed and its latency decreased in a manner similar to that seen with Pip citrate in bicuculline plus C_6 solution (compare Figure 7a and 5a; note also reduction in the amplitude of the spike after-

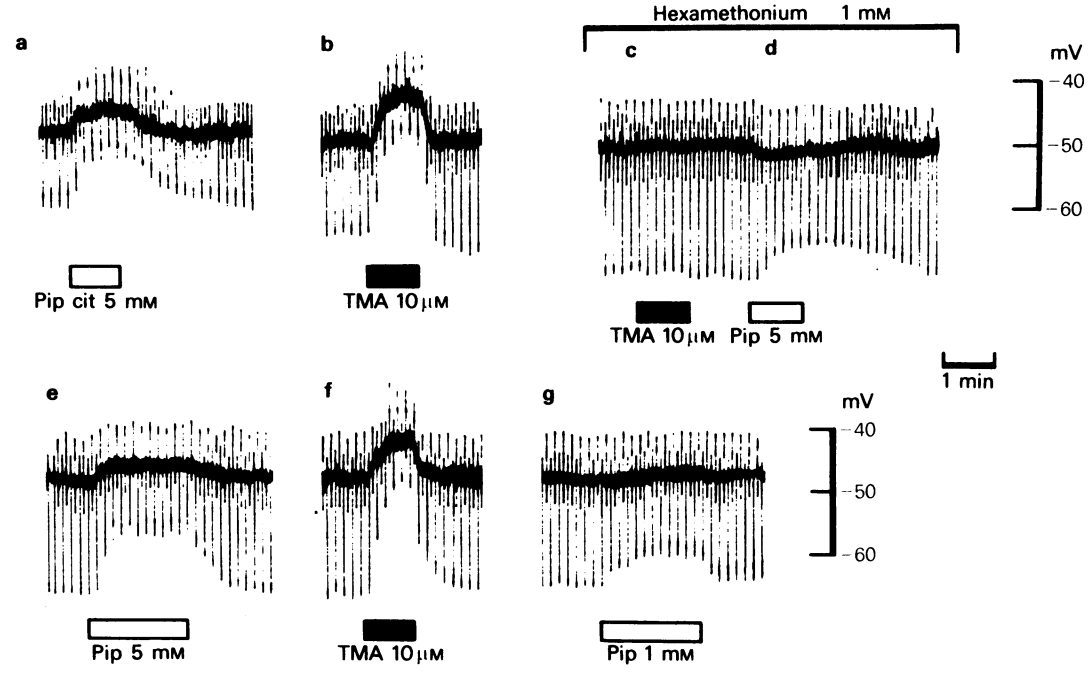

Figure 6 (a–g). Chart record of membrane potential and hyperpolarizing electrotonic potentials (evoked by −0.3 nA; 300 ms duration). (a, b). Control responses to piperazine (Pip) citrate and tetramethylammonium bromide (TMA); (c,d) residual responses to TMA and Pip citrate in C_6 1 mM; (e, f) recoveries. A response to a lower dose of Pip citrate is included in (g) for comparison.

hyperpolarization). A comparable, though more slowly developing effect on the cell was produced by perfusing with a Ca^{2+}-free solution (Figure 7b). The effects of applying Na citrate or lack of Ca^{2+} were fully reversed in control Krebs solution, and were not mimicked by applying a steady d.c. current to the cell in order to evoke an equivalent hyperpolarization.

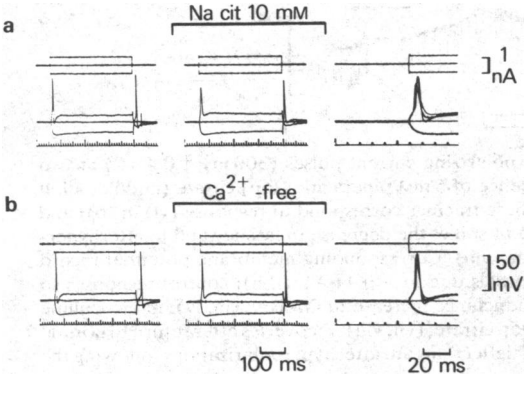

Figure 7 Response to depolarizing and hyperpolarizing current pulses (± 0.6 nA; 300 ms; cf. Figure 4 legend). Row (a) shows effect of 10 mM Na citrate on a single cell. The superimposed spikes on the right show the progressive change in spike latency and amplitude at faster sweep. Row (b) shows the similar effect produced by a Ca^{2+}-free medium in the same cell.

Discussion

Our results domonstrate that Pip, in relatively high concentrations, depolarized rat sympathetic neurones and increased their input conductance. These responses were most probably mediated by nicotinic cholinoceptors since they were fully blocked by C_6. No clear evidence for an action of Pip on ganglionic GABA receptors was seen. In fact, the small component of the Pip response sensitive to bicuculline or picrotoxin (cf. Figure 3) was probably due to an action of these antagonists on cholinoceptors themselves (Bowery & Brown, 1974; Marder & Paupardin-Tritsch, 1980); the characteristics of the Pip response (including its ionic dependence) also argue against a GABA agonist activity. This latter finding contrasts with previous data on crustacean muscle (Iravani, 1965; Constanti & Nistri, 1976) where Pip has a picrotoxin-sensitive, GABA-mimetic action. Such a sharp difference in GABA receptor sensitivity to Pip confirms our previous proposal that there are at least two types of GABA receptors probably present on these preparations (Nistri & Constanti, 1979).

The cholinomimetic effect of Pip was selective for nicotinic receptors, since hyoscine, a potent muscarinic antagonist (Brown *et al.*, 1980), did not affect Pip responses. Moreover, there was no residual effect of Pip hexahydrate in C_6 solution. We also surmise

that Pip is probably more active on nicotinic receptors in ganglia than those in skeletal muscle since Pip has no agonist or antagonist actions on frog muscle (Nistri & Arenson, 1978).

It is not immediately obvious from the chemical structure of Pip how such a cyclic molecule could interact with nicotinic receptors. However, pharmacological studies with closely related cyclic amines, piperidine and pyrrolidine, indicate that these agents also mimic the action of nicotine or acetylcholine on peripheral and central nervous systems (Kasé *et al.*, 1967; Kasé, Miyata, Kamikawa & Kataoka, 1969). Another possibility is that Pip might release endogenous acetylcholine from rat ganglia; although this was not tested directly, it seems rather unlikely since no release-promoting activity of Pip was found in spinal neurones (indeed, Pip was reducing acetylcholine release here; Nistri & Arenson, 1978). In the present experiments, any significant acetylcholine release would presumably evoke both nicotinic and muscarinic effects, yet the action of Pip was unchanged in the presence of the muscarinic antagonist, hyoscine.

From our results on ganglia, it would seem premature to suggest that the main neurological side-effects of Pip are attributable to stimulation of central nicotinic receptors. When the effects of Pip and piperidine on brain synaptic transmission and EEG were compared in the cat (Kasé *et al.*, 1969), piperidine had predominantly excitatory effects, followed by depression (like nicotine or acetylcholine), whereas Pip mainly induced neuronal depression (cf. also Shinozaki & Konishi, 1970). Nevertheless, our findings raise the possibility that a nicotinic action of Pip on some mammalian neurones contributes to some of the side-effects of the drug. Whether this nicotinic effect is superimposed on a GABA-like action on brain neurones (but not neurones in ganglia or the spinal cord, cf. Constanti & Nistri, 1976) remains a question for further experiments.

Although the concentrations of Pip used in the present study may seem high, the concentrations active on *Ascaris* (Del Castillo *et al.*, 1964) or crustacean muscle (Iravani, 1965; Constanti & Nistri, 1976) were also in the mM range. Unfortunately, we are unaware of published data on plasma levels of Pip after oral administration to man; the only measurements reported have been on urinary levels (Hanna & Tang, 1973). Pip is readily absorbed from the small intestine (Katz, 1977) and between 15–75% of a dose may be recovered unchanged in the urine. In view of the rather large recommended dose of Pip (4 g), it is not unreasonable to expect plasma concentrations near the range found effective in our experiments.

Finally, we suggest due care with the use of the Pip citrate salt in pharmacological experiments since chelation of extracellular Ca^{2+} by citrate was capable of producing distinct changes in spike configuration and neuronal excitability. Such changes probably reflect interference with Ca^{2+}-dependent components of the spike (McAfee & Yarowsky, 1979), and with the surface charge density of the neuronal membrane with a consequent increase in excitability (Frankenhaeuser & Hodgkin, 1957; Krnjević, Lamour, MacDonald & Nistri, 1979).

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