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A comparison of the local anaesthetic-, "quinidine-like"- and adrenergic β-blocking-activities of five β-receptor antagonists

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Propranolol, pronethalol, INPEA, MJ1999 and MJ1998 exhibit local anaesthetic activity when assessed by infiltration anaesthesia and at motor nerve endings but only propranolol, pronethalol and INPEA exhibit local anaesthetic activity on the phrenic motor nerve. All the β-adrenergic blocking agents exhibit a "quinidine-like" activity and there appears to be a close correlation between local anaesthetic and "quinidine-like" activities, which cannot be extended to include adrenergic β-receptor antagonism.

Dawes (1946) observed a close correlation of the local anaesthetic and "quinidine-like" activities of a series of secondary and tertiary amines. The secondary amine β-adrenergic receptor antagonists, propranolol (Morales Aguilera & Vaughan Williams, 1965) and pronethalol (Gill & Vaughan Williams, 1964) are reported to exhibit local anaesthetic activity, whilst INPEA (Somani & Lum, 1965; Schmild & Hanna, 1967; Levy 1968), MJ1999 (Lish, Weikel & Dungan, 1965; Levy, 1968) and MJ1998 (Lish & others, 1965) are reported to be devoid of such activity. The local anaesthetic, "quinidine-like" and sympathetic β-receptor blocking activities of the β-receptor antagonists were measured in order to relate their local anaesthetic and "quinidine-like" actions and to consider whether this correlation itself could be related to adrenergic β-receptor antagonism.

EXPERIMENTAL

Local anaesthetic activity

Local anaesthetic action was measured by the intradermal weal method (Bülbring & Wajda, 1945) and by nerve conduction in the phrenic nerve (Matthews, 1961) and also at the neuromuscular junction of the isolated rat phrenic nerve diaphragm preparation (Bülbring, 1946; Straughan, 1961).

Intradermal weal method

Groups of six albino male guinea-pigs were used for the intradermal weal tests. The hair was removed from the test area on the back with a depilator of composition: 200 g barium sulphide in a 200 ml suspension containing 10% Teepol and 10% glycerin. 24 h after depilation, the local anaesthetic activities of the adrenergic β-receptor antagonists were compared with those of procaine using a 2 × 2 double blind, latin square assay design. Drugs were dissolved in saline, adjusted to pH

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7·10, and injected intradermally in volumes of 0·2 ml. Using a needle, the minimal force required to elicit a response was found and this stimulus was applied in a series of six tests on each test area at zero time and at intervals of 10 min for a 40 min period. A positive score was noted each time the guinea-pig did not respond.

Conduction studies in the isolated phrenic nerve diaphragm preparation

A triangular segment of the left hemidiaphragm with attached rib segments, intercostal tissue and phrenic nerve of a decapitated rat was attached by its intercostal margin to a steel holder, which acted as an electrode for direct muscle stimulation, and by a fine steel wire to the central tendon, which served to attach the diaphragm to a semi-isotonic writing lever. The preparation was bathed in Krebs solution at 37·0° gassed with 5% carbon dioxide in oxygen at pH 7·32. For studies on nerve conduction, the cut end of the phrenic nerve was drawn into a nerve bath 0·3 ml capacity, through a small perforation in a rubber dam closure at one end, thus separating the fluid in the nerve bath from that bathing the diaphragm. The nerve was applied to two platinum electrodes in the nerve bath. To study the effects of drugs on transmission at the neuromuscular junction, the phrenic nerve was placed around electrodes in the diaphragm bath.

The local anaesthetic activities of the adrenergic β-receptor antagonists were compared with those of procaine using a 2 × 2 latin square design. The drug contact time was 3 min, direct muscle stimulation was applied for 20 s at zero, one and 3 min and the cycle time was 20 min. The diaphragms were stimulated indirectly through the phrenic nerve at a frequency of 15 impulses/min with rectangular pulse width of 0·1 ms at a supramaximal voltage and directly with rectangular pulse width of 1·0 ms at a supramaximal voltage. Before an assay, a control dose of procaine was repeatedly administered, using the experimental contact and cycle times, until the responses were constant.

Mode of action at the neuromuscular junction

The actions of the adrenergic β-receptor antagonists were compared with those of procaine and tubocurarine using a method devized by Straughan (1961). The diaphragm was stimulated indirectly through the phrenic nerve with rectangular pulses of 0·1 ms duration at a supramaximal voltage at a frequency of 15 impulses/min. The drug contact time was 3 min and the cycle time 20 min. A concentration of drug was found which produced a 5–20% inhibition of the indirectly elicited diaphragm response. The procedure was then repeated with a lower concentration of drug to find the highest concentration which had no effect on the response of the diaphragm. The preparation was then equilibrated for 10 min with $2{\cdot}5 \times 10^{-7}$ g/ml eserine sulphate and the subeffective dose was then added to the bath and its effect noted.

"Quinidine-like activity"

The "quinidine-like" activity of the adrenergic β-receptor antagonists was determined as a measure of the reduction in the maximal drive rate, using the isolated atria preparation (Dawes, 1946).

Guinea-pig atria were suspended in an organ bath containing Locke solution at 29° aerated with 5% carbon dioxide in oxygen at pH 6·8. The gas mixture passed

into the organ bath up a Perspex tube, in the upper end of which were two silver stimulating electrodes. The tip of the right atrium was pulled into the tube to make contact with the electrodes and the left atrium was connected to a strong spring lever which wrote on a smoked kymograph.

Rectangular pulse waves of 3·0 ms duration at double the voltage threshold determined at 240 beats/min were used. The frequency of stimulation was variable over a range of 120–414 beats/min. The atria were stimulated for 10 s and rested for 15 s periods. The frequency of stimulation was increased in steps of 6 impulses/min until the frequency at which the atria could not follow was ascertained. The maximal drive rate of the atria was taken as the highest frequency that the atria could follow. When the maximal drive rate had been confirmed, the test drug was added to the bath for 10 min and then the maximal drive rate of the atria was again measured.

Adrenergic β-receptor antagonism

The adrenergic β-blocking activities of the drugs were assessed by their antagonism of the response of the rabbit isolated ileum preparation to isoprenaline (Lockett & Bartlet, 1956).

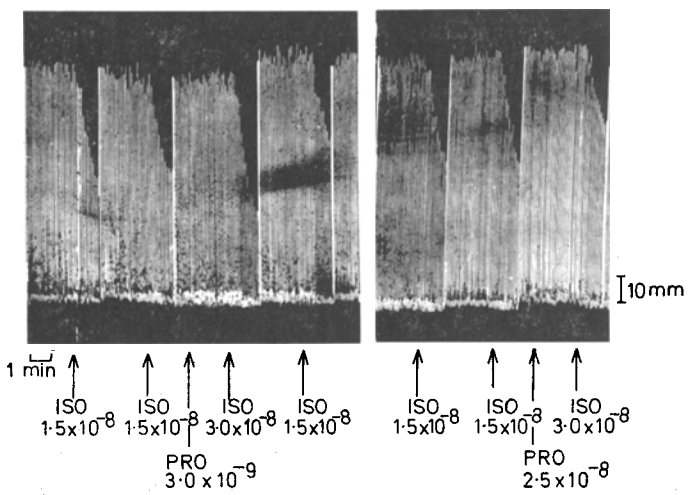

FIG. 1. Rabbit isolated ileum preparation. The tracing shows the effects of isoprenaline sulphate (ISO), on the spontaneous contractions of the ileum, and the antagonism of the isoprenaline response with propranolol hydrochloride (PRO). The tracing illustrates the method of determining pA_2 values. See text for explanation. The preparation was bathed in Krebs solution at 38·0° containing phentolamine ($5·0 \times 10^{-8}$ g/ml). All concentrations are expressed as the salt in g/ml.

Approximately 1 inch of proximal ileum was suspended in an organ bath containing Krebs solution oxygenated with 5% carbon dioxide in oxygen of pH 7·32. Phentolamine ($5·0 \times 10^{-8}$ g/ml) was included in the Krebs solution to prevent possible inhibition of spontaneous contractions due to α-receptor stimulation. This concentration of phentolamine was that found to completely abolish a 50% reduction in response produced by phenylephrine. Spontaneous contractions were recorded on a smoked kymograph with a frontal writing lever which magnified 8 times.

Control responses to a concentration of isoprenaline which produced approximately a 50% reduction (K) of the spontaneous contractions, were obtained using

a 1 min contact time for isoprenaline and a 5 min cycle time. The adrenergic β-receptor antagonist was then added to the bath and acted for 2 min, at which time twice the previous dose of isoprenaline was added to the bath and the response (2K) noted (Fig. 1).

RESULTS

Local anaesthetic activity

Propranolol, pronethalol, INPEA, MJ1999, MJ1998 and procaine showed local anaesthetic activity by the intradermal weal method. The potency ratios and confidence limits (Finney 1952), are shown in Table 1.

Propranolol, pronethalol, INPEA, MJ1999, MJ1998 and procaine also block conduction at the neuromuscular junction of the phrenic nerve diaphragm. The mode of action of the drugs was ascertained using an anticholinesterase-treated preparation. A normally sub-effective concentration of tubocurarine had no effect, but a normally subeffective concentration of procaine reduced the indirectly-elicited responses of the eserinized phrenic nerve diaphragm to the pre-eserinization level of response. Normally sub-effective concentrations of the adrenergic β-receptor antagonists acted in a similar manner to procaine, demonstrating their procaine-like nerve blocking action. The potencies of the drugs in producing nerve blockade at the neuromuscular junction are of the same order as their potency when assessed by the intradermal weal method, but the concentrations required at the neuromuscular junction are much smaller than those required for local anaesthesia by the intradermal weal method (Table 1).

Table 1. *Local anaesthetic activity assessed by the intradermal weal method and at motor nerve terminals*

	Intradermal weal method			At motor nerve terminals	
Drug	Effective concentration g/ml	Potency expressed against procaine (= 100)	Confidence limits $P = 95{\cdot}0\%$	Effective concentration g/ml	Potency expressed against procaine (= 100)
Propranolol hydrochloride	$2{\cdot}5 \times 10^{-3}$ to $5{\cdot}0 \times 10^{-3}$	198·0	112·0 to 240·0	$2{\cdot}5 \times 10^{-5}$ to $1{\cdot}0 \times 10^{-4}$	217·0
Pronethalol hydrochloride	$2{\cdot}5 \times 10^{-3}$ to $5{\cdot}0 \times 10^{-3}$	162·0	107·0 to 229·0	$2{\cdot}5 \times 10^{-5}$ to $1{\cdot}0 \times 10^{-4}$	168·0
INPEA hydrochloride	$2{\cdot}0 \times 10^{-2}$ to $4{\cdot}0 \times 10^{-2}$	21·6	18·5 to 26·4	$1{\cdot}0 \times 10^{-4}$ to $2{\cdot}5 \times 10^{-4}$	31·0
MJ 1999 hydrochloride	$5{\cdot}0 \times 10^{-2}$ to $1{\cdot}0 \times 10^{-1}$	12·7	7·6 to 17·4	$2{\cdot}5 \times 10^{-4}$ to $5{\cdot}0 \times 10^{-4}$	13·6
MJ 1998 hydrochloride	$5{\cdot}0 \times 10^{-2}$ to $1{\cdot}0 \times 10^{-1}$	6·3	4·1 to 8·4	$5{\cdot}0 \times 10^{-4}$ to $1{\cdot}0 \times 10^{-3}$	7·6

* INPEA; *N*-isopropyl-*p*-nitrophenylethanolamine. MJ 1999; 4-(2-isopropylamino-1-hydroxyethyl)methanesulphonanilide. MJ 1998, 4-(2-methylamino-1-hydroxypropyl)methanesulphonanilide.

Propranolol (minimal effective concentration $1{\cdot}0 \times 10^{-4}$ g/ml), pronethalol ($1{\cdot}0 \times 10^{-4}$ g/ml), INPEA ($5{\cdot}0 \times 10^{-4}$ g/ml) and procaine ($1{\cdot}0 \times 10^{-4}$ g/ml) block conduction along the phrenic nerve of the phrenic nerve diaphragm preparation, but MJ1999 and MJ1998 are devoid of such local anaesthetic activity in concentrations up to $5{\cdot}0 \times 10^{-2}$ g/ml, even when the contact time is extended to 15 min.

"Quinidine-like" activity

All the adrenergic β-receptor antagonists exhibited "quinidine-like" activity on the electrically driven isolated atria preparation. The "quinidine-like" activity was measured as a % reduction of the maximal drive rate of the atria which was determined as:

$$\frac{\text{The difference in the maximal drive rate before and after the drug} \times 100}{\text{The last determined maximal drive rate before the drug action}}$$

Four determinations were made at each of four doses and the relative activities assessed graphically, the point of comparison being taken where the maximal drive rate was reduced by 20%. The relative activities of the adrenergic β-receptor antagonists compared with procaine as "quinidine-like" agents were similar to their potencies as local anaesthetic agents when assessed by the intradermal weal method and as nerve blocking agents at the neuromuscular junction. The concentrations required to produce "quinidine-like" activity were similar to those required to produce nerve block at the neuromuscular junction (Table 2).

Table 2. *The "quinidine-like" activity of the adrenergic β-receptor antagonists assessed on the isolated guinea-pig atrial preparation*

Drug	Effective concentration g/ml	Potency of drugs expressed against procaine (= 100)
Propranolol hydrochloride	$2{\cdot}5 \times 10^{-6}$ to $1{\cdot}0 \times 10^{-5}$	206·0
Pronethalol hydrochloride	$2{\cdot}5 \times 10^{-6}$ to $1{\cdot}0 \times 10^{-5}$	170·0
INPEA hydrochloride	$2{\cdot}0 \times 10^{-5}$ to $4{\cdot}0 \times 10^{-5}$	45·5
MJ 1999 hydrochloride	$5{\cdot}0 \times 10^{-5}$ to $1{\cdot}0 \times 10^{-4}$	15·6
MJ 1998 hydrochloride	$1{\cdot}4 \times 10^{-4}$ to $2{\cdot}0 \times 10^{-4}$	6·8

Adrenergic β-receptor antagonism

All the adrenergic β-receptor antagonists inhibit the effects of isoprenaline on the rabbit isolated ileum preparation. Using four determinations at five doses for each drug, the relative potencies were measured graphically from a log dose against response $(2K-K)/K \times 100$, where 2K and K were measured as a % reduction in the spontaneous contractions of the ileum produced by isoprenaline. The point

of comparison was taken where $(2K - K)/K \times 100 =$ zero, at which point $2K = K$. The potencies of the drugs were expressed against propranolol (= 100) and also as pA_2 values (Table 3). The concentrations of adrenergic β-receptor antagonists required to produce adrenergic β-blockade are some 1000 times smaller than the concentrations required to produce a "quinidine-like" action on the atria and blockade at motor nerve endings.

Table 3. *Adrenergic β-receptor antagonism assessed by inhibition of the response of rabbit ileum to isoprenaline*

Drug	Effective concentrations g/ml	Potency expressed against propranolol (= 100)	pA_2 values
Propranolol hydrochloride	$3{\cdot}125 \times 10^{-9}$ to $5{\cdot}0 \times 10^{-8}$	100·0	7·27
Pronethalol hydrochloride	$2{\cdot}5 \times 10^{-8}$ to $2{\cdot}0 \times 10^{-7}$	19·6	6·58
INPEA hydrochloride	$2{\cdot}5 \times 10^{-8}$ to $8{\cdot}0 \times 10^{-7}$	7·7	6·11
MJ 1999 hydrochloride	$1{\cdot}25 \times 10^{-8}$ to $2{\cdot}0 \times 10^{-7}$	19·6	6·70
MJ 1998 hydrochloride	$2{\cdot}0 \times 10^{-7}$ to $1{\cdot}6 \times 10^{-6}$	1·40	5·34

DISCUSSION

All the adrenergic β-receptor antagonists examined here exhibited local anaesthesia by the intradermal weal (infiltration anaesthesia) method. The ratio of activities of propranolol : procaine 1·98 : 1·00, and pronethalol : procaine 1·65 : 1·00, as local anaesthetics are similar to those reported for propranolol : procaine 2·30 : 1·00 (Morales Aguilera & Vaughan Williams, 1965), and pronethalol : procaine 1·80 : 1·00 (Gill & Vaughan Williams, 1964), using the same method and animal species. The demonstration that MJ1999 and MJ1998 exhibit local anaesthesia in concentrations of $5{\cdot}0 \times 10^{-2}$ g/ml and greater, are not in agreement with the observations of Lish & others (1965) who reported that MJ1999 and MJ1998 were devoid of local anaesthetic activity in doses up to $6{\cdot}2 \times 10^{-2}$ g/ml when assessed by the intradermal weal method, or with the findings of Levy (1968), who used the rabbit corneal method, or Schmild & Hanna (1967) who used earthworms as the diagnostic agent for assessing local anaesthetic activity. Contrary to the findings of Somani & Lum (1965) and Levy (1968) who used the corneal method and Schmild & Hanna (1967), who used earthworms, and who reported INPEA to be devoid of local anaesthetic activity, the intradermal weal method showed INPEA to have one-third the activity of procaine. These differences, not unexpected, are to be attributed to the known influence of routes of absorption.

The ratio of activities of the adrenergic β-receptor antagonists ascertained by blockade of neuromuscular transmission, which was demonstrated to be a procaine-like local anaesthetic action, are very similar to the values obtained by the intra-

dermal weal method. The *in vitro* nerve experiments show propranolol, pronethalol and INPEA to block conduction in the phrenic nerve whilst MJ1999 and MJ1998 did not. Calculated from the partition coefficients obtained for a water–chloroform suspension, propranolol (97%), pronethalol (93%) and INPEA (56%) have high lipid solubilities, while MJ1999 (3·0%) and MJ1998 (3·5%) (Levy, 1968, Larsson, personal communication), have low lipid solubilities. The lack of effect of MJ1999 and MJ1998 on nerve conduction in the phrenic nerve may be explained in terms of the low lipid solubilities of these compounds, rendering them unable to penetrate the highly lipid nerve and myelin sheaths. At the fine diameter nerve terminals of sensory and motor nerves, MJ1999 and MJ1998 are able to reach the cell membrane and produce their local anaesthetic effects.

All the adrenergic β-receptor antagonists exhibit a "quinidine-like" action on the electrically driven atria preparation. A comparison of the local anaesthetic and "quinidine-like" activities of the adrenergic β-receptor antagonists with those of procaine hydrochloride showed their relative potencies as local anaesthetic and "quinidine-like" agents to be similar. Dawes (1946) also found the amines with the most potent local anaesthetic activity were most potent as "quinidine-like" agents. Levy (1968), who introduced the additional factor of corneal penetration with the rabbit corneal method, could not demonstrate a correlation between the local anaesthetic and "quinidine-like" activities of some adrenergic β-receptor antagonists.

The potency of the drugs as β-receptor antagonists was propranolol>pronethalol = MJ1999>INPEA>MJ1998. The correlation of local anaesthetic and "quinidine-like" activity cannot be extended to include adrenergic β-receptor antagonism because the relative activities of the drugs as β-receptor antagonists differ from their activities as local anaesthetic and "quinidine-like" agents, and the concentrations of these drugs required to produce their local anaesthetic and "quinidine-like" activities are some 1000 times greater than those required to produce adrenergic β-blockade.

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