Assessment of two *in vitro* methods for determination of the apparent affinities of β-sympathomimetics on myocardial contractility

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As long ago as 1871 it was reported by Bowditch that changes in heart frequency influences the degree of contractility of isolated myocardial preparations. This was subsequently confirmed by Koch-Weser & Blinks (1963). It has recently been shown that quantitative differences exist between structure-activity relation for chronotropic and inotropic effects of β-adrenoceptor stimulants and β-sympatholytics and it was postulated that the cardiac β_1-adrenoceptors could probably be subdivided into two groups (Dreyer & Offermeier, 1975). For quantitative *in vitro* assessments of apparent affinity values on the inotropic β_1-receptors it would be preferable to employ methods which could exclude, as far as possible, factors which may influence the results in one way or another.

The purpose of this investigation was to determine whether changes in frequency of contraction would influence the apparent affinities of different β-sympathomimetic drugs for the inotropic β-receptors to the same extent. To demonstrate that frequency of contraction does in fact influence the force of contraction, isolated left atrial strips from guinea-pigs were mounted in an organ bath perfused by a modified Locke solution at 37° gassed with 5% CO_2 in oxygen. A driving stimulus was applied through a pair of silver electrodes in contact with the left atrial strip. The strips were stimulated at different frequencies by square-wave pulses of 5 ms duration. Stimulus intensity was kept constant at approximately 50% above threshold.

Fig. 1 indicates that an increase in frequency of stimulation leads to an increase in the force of contraction up to a certain limit. Frequencies between 0·5 and 5 stimuli s^{-1} may be regarded as the physiological range.

To determine to what extent frequency of contraction influences the apparent affinities of β-sympathomimetic drugs for the inotropic receptors, we recorded changes in the force of contraction induced by different β-sympathomimetic drugs employing spontaneously beating atrial preparations (heart frequency 105 ± 10) and left atrial strips which were electrically stimulated at 1 s intervals. Full concentration-effect curves were obtained with the drugs employed and pD_2-values were calculated according to the method of van Rossum (1963).

The concentration-effect curves obtained with isoprenaline (a non-selective β-adrenergic stimulant), salbutamol (a β_2-selective stimulant, Brittain, Farmer & others, 1968) and dobutamine (an inotropic selective β-stimulant, Tuttle & Mills, 1975) using the two different methods, are presented in Fig. 2. The log concentration-effect curves obtained with the electrically stimulated preparations were in all cases to the right of those obtained with the spontaneously beating preparations. Comparison of the pD_2-values obtained by means of these two different methods, indicates that the relative apparent affinities for all three drugs tested were notably higher in the case of the spontaneously beating atria

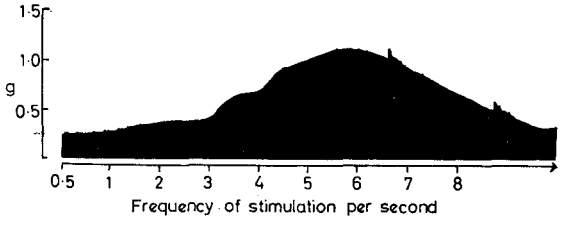

FIG. 1. Influence of frequency of stimulation per second on force of contraction (g) in electrically stimulated isolated left atrial strips.

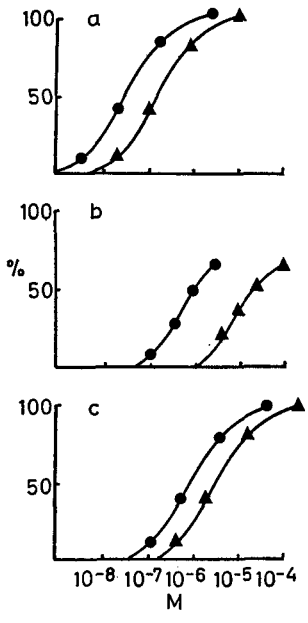

FIG. 2. Comparison of the inotropic effects of a-isoprenaline, b-salbutamol and c-dobutamine obtained with isolated atria (●) and electrically stimulated left atrial strips (▲). (The maximal increase in contractility (%) obtained with isoprenaline was taken as 100%).

* Correspondence.

Table 1. *Comparison of the apparent inotropic affinities of isoprenaline, salbutamol and dobutamine obtained with spontaneously beating atria* vs *electrically stimulated left atrial strips of the guinea-pig.*

Compound	Strip* pD_2 values	Atria† pD_2 values	RA‡ strip	RA‡ atria	*P*
Isoprenaline	6·8 ± 0·2	7·5 ± 0·2	100	500	<0·01
Salbutamol	5·1 ± 0·3	6·3 ± 0·8	2	30	<0·005
Dobutamine	5·5 ± 0·3	6·1 ± 0·3	5	15	<0·1

* Electrically stimulated.
† Spontaneously beating.
‡ Relative pD_2-value of isoprenaline on left atrial strip taken as 100.
RA—relative affinities.

(see Table 1). The higher apparent affinities obtained in the case of the spontaneously beating preparations may be ascribed to the fact that the drugs also increased the frequency of contraction which potentiated the force of contraction.

From our results we could conclude that changes in frequency of contraction do influence apparent affinity values for the inotropic receptors for β-adrenergic agonists to varying degrees. Since affinities for chronotropic and inotropic β_1-adrenoceptors may differ, the influence of changes in frequency on the apparent affinity for the inotropic receptors may vary from one drug to the next. Since β-adrenergic agonists may be chronotropic or inotropic selective, their chronotropic effects may influence the determination of relative inotropic affinities to varying degrees when spontaneously beating *in vitro* preparations are used for the assessment of the inotropic effects.

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A benzimidazole derivative (7110 MD) with gastric antisecretory and antiulcer activity

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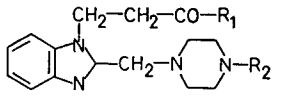
The compound 7110 MD 1-(2-benzoyl ethyl) 2-(cinnamyl piperazinyl 1-methyl) benzimidazole dimaleate belongs to a series of benzimidazole derivatives of general structure (I) (Fauran, Eberlé & others, 1972) which possess gastric antisecretory and antiulcer properties.



I

7110 MD

$R_1 = C_6H_5$ $R_2 = CH_2-CH=CH-C_6H_5$

The compound shows notable gastric antisecretory activity (Table 1). Administered orally, intravenously (i.v.) or intraduodenally (i. d.) to the 4 h pylorus-ligated rats, it decreases the volume and acid concentration of gastric secretion at doses well below the LD50. The large difference between the LD50 doses for the oral and intravenous routes is not the result of poor absorption from the gut because the ED50 values for gastric secretion are low for both routes.

* Correspondence.

In the urethane anaesthetized rat (1·75 g kg^{-1}, i. m.), gastric secretion stimulated by carbachol 5 μg kg^{-1} h^{-1} (i. v.) is reduced to 53% by the compound at 1·50 mg kg^{-1} (i.v.) and gastric secretion stimulated by pentagastrin 2 μg kg^{-1} h^{-1} (i.v.) is reduced by 50% at 3 mg kg^{-1} (i.v.), but hypersecretion induced by histamine 0·69 mg kg^{-1} h^{-1} (i.v.) is not modified at 10 mg kg^{-1} (i.v.). The antagonism of 7110 MD towards pentagastrin 2 μg kg^{-1} h^{-1} (i.v.) was confirmed in one conscious dog with a Heidenhain pouch: at 2 mg kg^{-1} (i.v.), the drug decreased gastric juice output by 53% and free acid output by 69%. This gastric antisecretory activity is accompanied by antiulcer properties since the drug given orally or intraduodenally prevents the formation of experimental ulcers of various origins (ED50 by the oral route ranging from 4·2 to 38 mg kg^{-1} and intraduodenally from 1·6 to 28·5 mg kg^{-1}, Table 2). Yet, even at high doses the drug has little effect on duodenal ulcer and none on the gastric ulcer due to histamine.

While the drug is active on the stomach *in vivo*, its anti-acetylcholine activity *in vitro* against acetylcholine-induced contractions in the rat duodenum is weak (50% inhibition at 22·5 mg ml^{-1}, 1/5000 of atropine). *In vivo*, 7110 MD has no intestinal spasmolytic properties in the