STANDARDISED ISOPRENALINE SENSITIVITY TESTS — A COMPARISON OF EXISTENT METHODS

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- 1 Six healthy subjects (four male, two female, aged 19–24 years) received intravenous boluses of isoprenaline sulphate by two standardised methods of injection (Cleaveland *et al.*, 1972; George *et al.*, 1972). Heart rate changes were analysed by three different methods.
- 2 The I_{25} (dose of isoprenaline required to increase the heart rate by 25 beats/min) was not significantly altered by either the method of injection or the method of heart rate measurement.
- 3 The slope of the heart rate dose response curve was significantly steeper when isoprenaline was injected in a fixed 2 ml volume through a three-way tap, and when the heart rate was measured from the shortest three R-R intervals.
- 4 However the differences in method of injection and heart rate measurement between the two techniques do not appear to produce major alterations in the results obtained, particularly the I_{25} .

Introduction

The blockade of β -adrenoceptors is commonly investigated by determining the shift to the right of a dose-response curve of the increase in heart rate elicited by bolus intravenous injections of isoprenaline. Two standardised methods have been described. Cleaveland *et al.* (1972) used a variable volume (< 1 ml) of isoprenaline hydrochloride injected into a fast flowing drip and measured peak heart rate from the three shortest R-R intervals of an electrocardiogram. They found the mean dose of isoprenaline (I_{25}) required to increase the heart rate by 25 beats/min was 1.02 μ g of base and the mean slope of the dose-response curve was 8.35. George *et al.* (1972) used a fixed volume (2 ml) of isoprenaline sulphate injected through a three-way tap and measured heart rate with either an instantaneous ratemeter or a digital recorder. A consideration of their results shows a mean I_{25} of between 2.28–3.04 μ g of base. Similarly the slope of their dose-response curves ranged from approximately 2.7 to 10.9. Hence, it appears that the method of George *et al.* (1972) resulted in a larger I_{25} with a tendency towards a shallower slope. The present study was designed to assess the effects of method of drug administration and mode of heart rate measurement on the assessment of isoprenaline sensitivity in young healthy subjects.

Methods

The study was approved by the University Ethical Committee.

Six healthy non-smoking volunteers (four male, two female; aged 19–24 years; weight 52–84 kg) were studied. Routine physical examination and standard twelve lead electrocardiogram were normal in all subjects.

All experiments were performed in a quiet room at the same time of the morning, after a light breakfast containing no caffeine. The subject rested supine for 30 min with head and shoulders supported. An intravenous line (Butterfly 19G) was established in the right antecubital fossa and patency maintained by sodium chloride 0.9% dripping slowly from a burette administration set (Buretrol). Subsequent intravenous injections of a range of isoprenaline sulphate concentrations previously prepared in sodium metabisulphite 0.1% preservative were flushed by 15 ml of sodium chloride from the burette. Lead II of the electrocardiogram was recorded on a Minigraph recorder (Cardiac Recorder Ltd.). The electrocardiogram was also monitored from chest leads using an instantaneous ratemeter (Devices ratemeter type 2750) and recorder (Devices MX4). Respirations were monitored from a chest strain gauge (Lectromed Transducer 4320) on the same recorder.

After several test doses to familiarise the subjects with the procedure, graded bolus injections of isoprenaline were administered by two techniques. The order of injection methods was randomised but, for each, at least five points were obtained on the dose-response curve.

Inject I A three-way tap was inserted between the administration set and the intravenous needle. The desired quantity of isoprenaline was drawn into a graduated 1 ml syringe and then carefully diluted with sodium chloride 0.9% up to 2 ml, in a 2 ml syringe. The injection was given rapidly through the three-way tap and immediately flushed by 15 ml of sodium chloride.

Inject II The desired quantity of isoprenaline was drawn into a graduated 1 ml syringe in a volume not exceeding 1 ml and injected directly and rapidly through the rubber tubing of the drip sleeve via a 27 g needle. Immediately prior to the injection the drip was increased to a fast flowing rate which was continued after the injection until 15 ml of sodium chloride had run through.

The electrocardiogram was recorded during a 45 s control recording period prior to isoprenaline injection

and from 30–75s post injection. It was analysed in three ways:-

R-R Maximum heart rate was calculated from the shortest three consecutive R-R intervals in limb lead II;

Counts In a 15 s period around this maximum heart rate, the number of individual QRS complexes were counted in limb Lead II;

Meter The maximum heart rate was read from the ratemeter recording of the chest leads.

For each method of injection regression lines could be drawn for the heart rate change from each method of heart rate measurement. From the regression equation the slope of the curve was recorded and the I_{25} calculated.

The results are shown as mean $\pm$ s.e. mean. They were statistically compared by two-way analysis of variance and Student's paired or unpaired *t*-test.

Results

Although the injection of isoprenaline could be given more rapidly by *Inject I*, there was no significant difference between the two injection methods in the

Figure 1 Simultaneous heart rate measurements (*R-R*, *Counts*, *Meter*) for each of six subjects, are shown by both methods of isoprenaline injection (*Inject I*, *Inject II*) at the dose of isoprenaline which most nearly produced a heart rate rise of 25 beats/min when heart rate was measured by *R-R*. Control and peak heart rates (beats/min) are shown with the mean $\pm$ s.e. mean.

* $P < 0.05$ when compared with corresponding value by *R-R*.

time to breathing change (I, 31.1 ± 1.0 ; II, 29.8 ± 1.0 s) or in the time to peak heart rate response (I, 50.4 ± 1.7 ; II, 52.4 ± 1.5 s). The variance around each regression line was not significantly different in any subject between the methods of injection for any method of heart rate measurement.

The resting control heart rate was 68.4 ± 1.1 beats/min when measured by *R-R*. This was significantly greater than when measured by *Counts* (64.1 ± 0.9 , $P < 0.01$) but significantly less than when measured by *Meter* (72.4 ± 1.2 , $P < 0.001$). Figure 1 shows the simultaneous heart rate measurements for each of the six subjects at the dose of isoprenaline administered which most nearly produced a heart rate rise of 25 beats/min when the increase was measured by *R-R*. When the heart rate was measured by *Counts* both control and peak values were less than *R-R*, with a tendency to underestimate peak heart rate to a greater extent. Measurement of heart rate by *Meter* tended to give a higher control heart rate than *R-R*, but the measured peak response appeared variable.

The dose-response curves of one representative subject for the increase in heart rate by each method of injection and each method of heart rate measurement are shown in Figure 2. The I_{25} values for each of the six subjects are shown in Table 1. The I_{25} was not significantly altered by either the method of injection or the method of heart rate measurement. The slopes

of the regression lines for each of the six subjects are shown in Table 2. The slope was significantly steeper when isoprenaline was given by *Inject I*. Within the same method of injection, measurement of heart rate by *R-R* tended to produce a steeper slope so that for *Inject I* the slope by *R-R* was significantly steeper than that by *Counts* ($P < 0.05$) and for *Inject II* the slope by *R-R* was significantly steeper than that by *Meter* ($P < 0.05$).

Inject I with heart rate measured by either *Counts* or *Meter* is the closest methodology to the description of George *et al.* (1972). *Inject II* with heart rate measured by *R-R* is the closest methodology to the description of Cleaveland *et al.* (1972). Table 3 shows that there were no significant differences in the I_{25} or slope for direct comparison of these methodologies.

Discussion

We have found that differences in the method of isoprenaline injection may cause different slopes in the heart rate dose-response curves but without significantly altering the I_{25} . *Inject I* (constant 2 ml volume injected through a three-way tap) produced a significantly steeper slope than *Inject II* (variable volume < 1 ml injected through drip sleeve). The former could be injected more rapidly but this did not result in a more rapid response as monitored by onset

Figure 2 Heart rate dose-response curves of one representative subject (RB), by each method of isoprenaline injection (*Inject I*, *Inject II*) and each method of heart rate measurement (*R-R* (●), *Counts* (▲), *Meter* (■))

Table 1 Dose of isoprenaline (μg) required to increase heart rate 25 beats/min (I_{25}), for each of six subjects, by method of heart rate measurement (*R-R*, *Counts*, *Meter*) and method of isoprenaline injection (*I*, *II*)

Subject	R-R		Counts		Meter	
	<i>I</i>	<i>II</i>	<i>I</i>	<i>II</i>	<i>I</i>	<i>II</i>
S F	0.57	0.54	0.57	0.50	0.62	0.64
M S	1.15	1.22	1.23	1.30	1.20	2.28
E H	0.85	1.03	0.83	1.22	0.95	2.02
P McG	1.24	1.79	1.57	1.71	1.92	2.19
G M	2.94	2.68	3.62	6.45	3.23	2.93
R B	0.51	0.51	0.91	0.88	0.56	0.48
Mean	1.21	1.30	1.46	2.01	1.41	1.76
s.e. mean	0.37	0.34	0.46	0.90	0.42	0.40
<i>P</i>	NS		NS		NS	

of breathing change or time to peak heart rate. The methods did not differ in the variability of the response as there was no significant difference between the variance from the regression lines and in each case five points were used. The difference is not likely to be an order effect as this was randomised. It might be postulated that the smaller concentrated volume with *Inject II* could have a more pronounced effect at the lower heart rates and therefore cause a shallower curve. However, the difference is in the opposite direction to the standardised descriptions where *Inject I* was used by George *et al.* (1972) who tended to get shallower curves than Cleaveland *et al.* (1972) who used *Inject II*.

Our results also show that the method of heart rate measurement may cause differences in the slope but not the I_{25} . Measurement of the heart rate by *R-R* gave a significantly steeper slope than measurement by *Counts* with *Inject I*, and than *Meter* with *Inject II*. Cleaveland *et al.* (1972) have previously shown that measurement of mean heart rate will result in a shallower curve as the baseline heart rate will be lower with an apparently greater increase following

isoprenaline, this being more important at the lower end of the dose-response curve. George *et al.* used either a digital recorder or an instantaneous rate-meter (Neilson). The specific digital recorder used (Emons & Conolly, 1971) gives a pen recording of the accumulated total of individual QRS complexes over a 15 s period. We calculated this manually from the ECG recording in a 15 s period around the peak heart rate as measured from the three shortest *R-R* intervals. Measurement of heart rate by *Counts* will tend to give a mean heart rate. We found the resting heart rate to be significantly lower with *Counts* than with *R-R* but also found that *Counts* tended to underestimate the peak response, and this may explain why it gives a shallower curve than measurement by *R-R*. As an instantaneous ratemeter, we used a Devices model and a pen recorder. With this we found resting heart rate was significantly higher than with *R-R* but the peak response tended to be more variable. This may suggest that *Meter* was the least suitable method of recording heart rate changes to isoprenaline.

We did not find a significant difference between our method which most closely resembled the

Table 2 Slope (beats min^{-1} ($\ln \mu\text{g}$) $^{-1}$) of heart rate dose response curves for each of six subjects by method of heart rate measurement (*R-R*, *Counts*, *Meter*) and method of isoprenaline injection (*I*, *II*)

Subject	R-R		Counts		Meter	
	<i>I</i>	<i>II</i>	<i>I</i>	<i>II</i>	<i>I</i>	<i>II</i>
S F	19.9	15.9	17.3	20.8	19.8	15.0
M S	23.3	16.8	19.4	12.1	22.7	8.2
E H	17.3	12.3	11.6	15.0	17.0	8.4
P McG	13.2	8.2	10.1	6.4	9.2	8.7
G M	14.2	12.5	14.5	5.2	14.7	8.1
R B	19.9	14.5	16.1	11.6	10.9	12.2
Mean	18.0*	13.4†	14.8	11.8	15.7	10.1
s.e. mean	2.0	1.3	1.4	2.3	2.1	1.2
<i>P</i>	< 0.01		< 0.05		< 0.001	

* $P < 0.05$ when compared to *Counts* by *Inject I*

† $P < 0.05$ when compared to *Meter* by *Inject II*

Table 3 A comparison of I_{25} (μg) and slope ($\text{beats min}^{-1} (\ln \mu\text{g})^{-1}$) for those methodologies most similar to the standardised descriptions of Cleaveland *et al.* (1972) and George *et al.* (1972). Each value is the mean $\pm$ s.e. mean ($n = 6$)

	Cleaveland <i>et al.</i> (1972)		George <i>et al.</i> (1972)
I_{25}	Inject II, R-R	1.30 $\pm$ 0.34	1.46 $\pm$ 0.46 Inject I, Counts
			1.41 $\pm$ 0.42 Inject I, Meter
Slope	Inject II, R-R	13.4 $\pm$ 1.3	14.8 $\pm$ 1.4 Inject I, Counts
			15.7 $\pm$ 2.1 Inject I, Meter

standardised description of Cleaveland *et al.* (1972) and our methods which were similar to that of George *et al.* (1972). Other variables, apart from method of injection or heart rate measurement, may have played a part. As each group used a different isoprenaline salt, we compared their I_{25} values as μg base. Though it is unlikely that the use of 5% dextrose as a flushing solution by Cleaveland *et al.* (1972) and 0.9% saline by George *et al.* (1972) would have been important, we gave the same volume of flushing solution, 15 ml of 0.9% saline, with every injection. Cleaveland *et al.* (1972) measured only three doses of isoprenaline while George *et al.* (1972) measured at least five. The increased number of points should result in a stronger correlation of the regression line but not an absolute difference unless they were on the flatter sections of the curve at the upper and lower ends. In our study five points were obtained on the straight part of the dose-response curve. It was possible to reproduce the injection method of George *et al.* (1972) exactly, but with Cleaveland *et al.* (1972) the size of syringe needle is not given. George *et al.* (1972) do not record the sex distribution of their subjects but Cleaveland *et al.* (1972) studied five males and five females and concluded there was no correlation of isoprenaline sensitivity with sex. Both groups concluded that variation in body weight made little difference to isoprenaline sensitivity.

It, therefore, appears that the differences between the results obtained in the descriptions of standardised methods of investigating isoprenaline sensitivity (Cleaveland *et al.*, 1972; George *et al.*, 1972) are not due to differences in methodology though these may influence the slope of the dose-response curve. Age differences between the two study populations may have been important. The ten subjects of Cleaveland *et al.* (1972) were young (aged 21–35 years) while the sixteen subjects of George *et al.* (1972) were older (aged 25–64 years), and it has been shown that sensitivity to isoprenaline decreases with age (Cleaveland *et al.*, 1972; Vestal *et al.*, 1978; Shocken & Roth, 1977; Doyle *et al.*, 1981). This present study used the same group of young subjects (aged 19–24 years) for each method and hence eliminated the effect of this variable.

The importance of an accurate reproducible test of isoprenaline sensitivity has been stressed previously (McDevitt, 1977). The present study investigated the extent to which the method of isoprenaline administration and mode of heart rate measurement affect the assessment of isoprenaline sensitivity. The results suggest that minor differences in methodology do not significantly alter the I_{25} though they may influence the slope of the heart rate dose-response curve.

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