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The isolated perfused heart preparation: two suggested improvements

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A new method of recording the contractions of the isolated perfused heart is described. By "transverse" recording, a signal of simple waveform is obtained from the right ventricle at constant diastolic tension. Changes in heart rate and force of contraction of the rat isolated perfused heart caused by (—)-noradrenaline were measured. Of the three methods of assessing the response: (1) the increment as a percentage of the control record, (2) the increment in absolute units and (3) the increment as a percentage of the maximum, the latter has most pharmacological meaning.

The isolated heart preparation, perfused by the Langendorf (1895) technique, is widely used to assess the activity of drugs which affect myocardial contractility and heart rate.

The method of making the record

The usual method of recording cardiac contractions uses a small metal clip attached to the apex of the heart. The rhythmical contractions are transmitted by a length of thread to either a spring loaded writing lever or a force displacement transducer ("longitudinal" recording Fig. 1a). While attempting to investigate the action of sympathomimetic drugs on the rat heart by this method, using a transducer and pen recorder, certain disadvantages became apparent:

(*a*) By virtue of the positioning of the clip, the signal produced is composed of the contractions of the left and right ventricles.

(*b*) A rotational component of the heart's contraction is very obvious in the freely

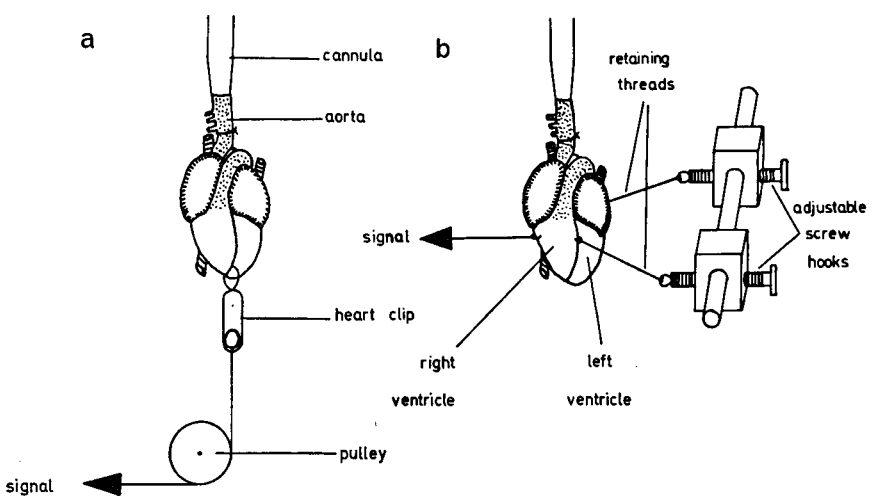

FIG. 1. a. "Longitudinal," b, "transverse" method of recording the contractions of the isolated perfused heart.

suspended preparation and this is added to the already compound signal from the heart clip.

(*c*) Instantaneous ratemeters for measuring heart rate and operational amplifiers for recording the rate of change of myocardial tension will only function accurately if the input signal has a *simple* waveform.

The problem of obtaining a homogenous signal from the contracting heart was overcome in the following manner. The heart was rapidly removed and suspended from a fine polyethylene cannula inserted into the aorta. This delivered warmed (37°), oxygenated (gassed with 5% carbon dioxide in oxygen) Krebs solution to the coronary circulation at a constant flow rate of 8 ml/min. Before securing the heart to the cannula it was rotated so that the right ventricle was facing an Ether 2 oz dynamometer (UF1). A stitch of terylene thread was inserted in the ventricular *septal* tissue on both the anterior and posterior surfaces of the heart approximately midway between the base and apex, and securely tied (Fig. 1b). Occlusion of any coronary vessels was carefully avoided. Each of the two threads was then tied to an adjustable screw hook mounted on a horizontal rod about 3 in from the heart and level with it so that the threads, when tightened, were horizontal and prevented any rotational movement of the contracting heart. A third stitch of terylene thread was inserted in the centre of the surface of the right ventricle in the same plane as the two retaining threads and passed to the dynamometer. This method of recording from the isolated heart was termed "transverse" recording. Alteration of the distance of the dynamometer from the heart by manipulation of a rackwork "X" block allowed fine control of the diastolic tension to which the right ventricle was subjected. A comparison of the records obtained with these two systems is shown in Fig. 2. The two records were obtained from the same heart at the same diastolic

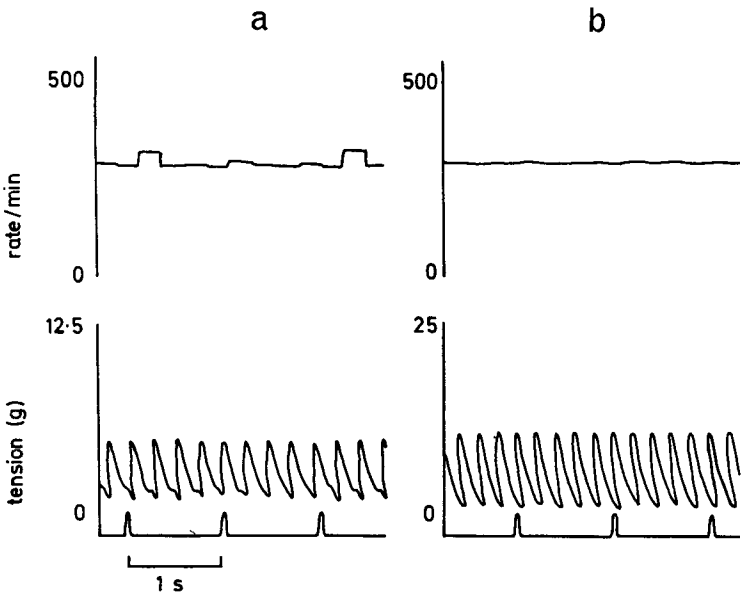

FIG. 2. A comparison of the records obtained by the methods of (a) longitudinal and (b) transverse recording of the contractions of the same rat isolated perfused heart. The diastolic tension was 1·5 g.

tension; a pure waveform is achieved by "transverse" recording with approximately twice the amplitude of the "longitudinal" record. Such a wave form has been successfully used as the signal for operational amplifiers measuring the rate of change of myocardial tension (differentiated record), measuring the area under the curve of the cardiac tension cycle over a predetermined time (integrated record), and also for instantaneous ratemeters.

Preliminary experiments using different diastolic tensions applied to the right ventricle revealed a very steep linear relation between diastolic tension and both the control force of contraction and the increment in force caused by a dose of noradrenaline. Since the diastolic tension alters spontaneously during the course of an experiment (usually it declines) it was readjusted to 1·5 g one min before each drug injection.

The method of presenting the results

The positive chronotropic and inotropic responses of the isolated heart preparation to injections of sympathomimetic drugs into the coronary circulation are expressed in one of two ways when attempting to assess the response to the drug quantitatively. Either the increment in rate or force of contraction caused by the drug is expressed as a proportion (%) of the magnitude of the parameter recorded immediately before drug injection (the commonest method, usually referred to as "percentage of control") or alternatively the size of this increment is given in absolute units. These two methods of presentation of results were compared on six rat isolated perfused hearts as follows. Doses of noradrenaline of 0·2, 1·0, 5, 50, 500, 1200 and 2400 ng were

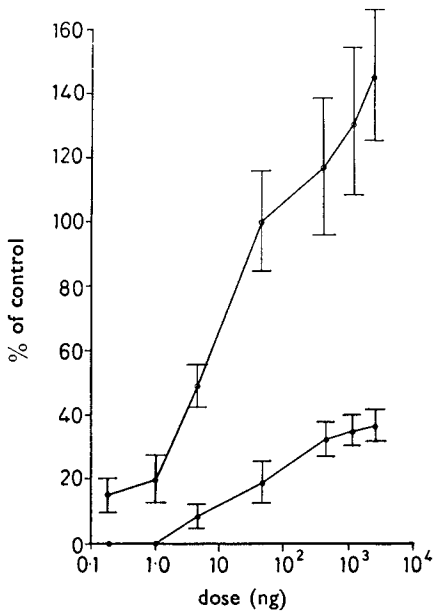

FIG. 3. Log dose response curves to (—)-noradrenaline on the rat isolated perfused heart preparation at a diastolic tension of 1·5 g. Heart rate = —●—●—. Right ventricular systolic tension = —○—○—. The response plotted is the maximum increment caused by noradrenaline expressed as a percentage of the heart rate or right ventricular systolic tension immediately preceding the injection. Each point is the mean of six experiments ± standard error.

injected in a volume of 0·05 ml into the aortic cannula of each rat heart, prepared for "transverse" recording. The dose sequence was randomized with a 10 min interval between doses.

When the results obtained with noradrenaline are presented as a percentage of the control value, the mean log dose response curves appear as in Fig. 3. Inspection of the records showed that the absence of a plateau in the tension log dose response curve is due not to an increased increment caused by the higher doses of noradrenaline but to an irreversible decline in the control tension record following these higher doses. The standard errors of the mean responses for most doses are large when presented in this way since they are compounded of three sources of variance: (i) that between hearts; (ii) that of regression of the increment of tension on the log dose noradrenaline in each heart; (iii) that of the magnitude of the control record which irreversibly declines after high doses and even in the untreated preparation is prone to spontaneous variation.

Since at constant diastolic tension the increment in tension produced by any dose of noradrenaline appeared to be independent of the control value there seemed no merit in including the size of this control value in the response presented.

When the results obtained with noradrenaline are presented as the increment in absolute units for the rate (beats/min) and force of contraction (tension in g), the mean log dose response curves for both now show a plateau still with large standard errors about the mean for any one dose (Fig. 4). When a constant dose of noradrenaline (ED50) was administered at 10 min intervals to one heart over a period

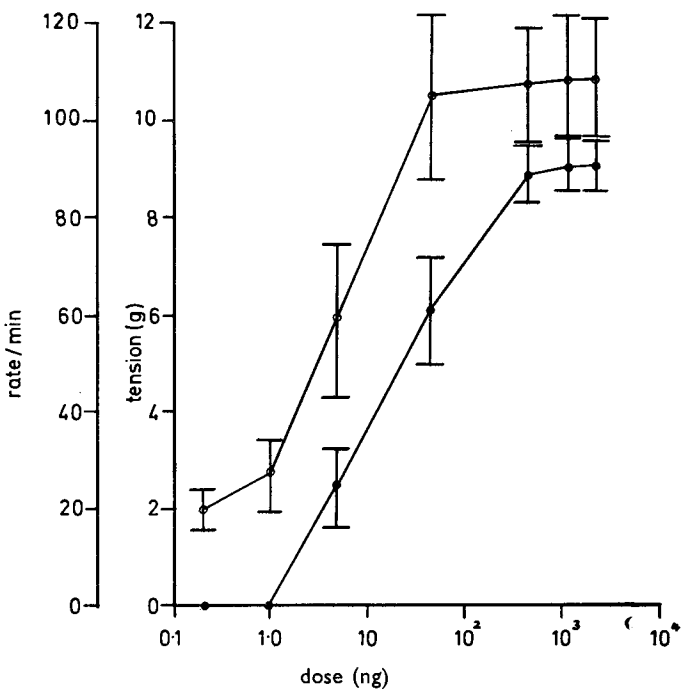

FIG. 4. Log dose response curves to (—)-noradrenaline on the rat isolated perfused heart preparation at a diastolic tension of 1·5 g. Heart rate = —●—●—. Right ventricular systolic tension = —○—○—. The response plotted is the maximum increment caused by noradrenaline in absolute units. Each point is the mean of six experiments ± standard error.

of 2 h the increment remained constant, so the large standard errors are indicative of the operation of two compounded sources of variance (i and ii above).

With other isolated tissues there are good theoretical reasons for the common practice of relating the response obtained with a dose of drug to the maximum response which may be achieved by the drug in that tissue. When the increments to noradrenaline are expressed in this way, the mean log dose response curves for each parameter (Fig. 5) have reduced variability compared with the previous method. It is felt that this represents the method of choice, only the variability between hearts remaining. The slope and position of the mean log dose response curves are not

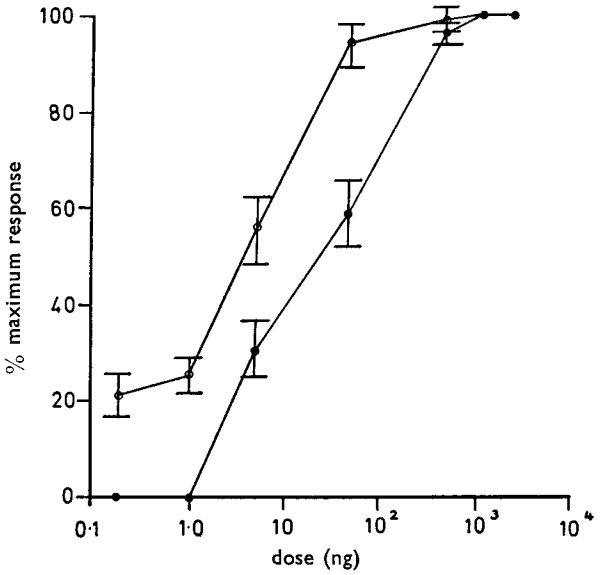

FIG. 5. Log dose response curves to (—)-noradrenaline on the rat isolated perfused heart preparation at a diastolic tension of 1·5 g. Heart rate = —●—●—. Right ventricular systolic tension = —○—○—. The response plotted is the maximum increment caused by noradrenaline expressed as a percentage of the increment caused by a dose of 2·4 μg noradrenaline. Each point is the mean of six experiments ± standard error.

significantly different at diastolic tensions of 1·5, 2·5, 3·5 and 4·5 g when the increment is calculated as a percentage of the maximum response. Thus, as long as the diastolic tension remains constant throughout a dose sequence, log dose response curves are comparable. An obvious further improvement to recording responses of the preparation by "transverse" recording with manual re-adjustment of the diastolic tension would be a servo-mechanism whereby the diastolic tension could be maintained at a constant preset value to minimise the errors resulting from the decline which is observed.

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