Short communications

Use of cumulative dose-response curves in potency comparisons of sympathomimetic amines on the cat soleus muscle

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The ability of β-adrenoceptor agonists to reduce the fusion of incomplete tetanic contractions of the soleus muscle of the cat has been used previously as a model to assess the potential tremor producing effect of sympathomimetic bronchodilators. The ability of (−)-isoprenaline, (−)-adrenaline, (±)-soterenol and (±)-quinterenol to depress incomplete tetanic contractions of the soleus has now been assessed using cumulative administration of the amines. The method quickly produced accurate and reproducible dose-response curves. It is particularly useful for evaluating the potency of long-acting compounds.

An unwanted effect of sympathomimetic bronchodilators is the production, in some patients, of a disturbing skeletal muscle tremor (Brittain, Jack & Ritchie, 1970; Legge, Gaddie & Palmer, 1971). It has been suggested (Bowman & Nott, 1970; Brittain *et. al.*, 1970), that the cat soleus nerve-muscle preparation provides a useful model for forecasting the likelihood of a tremor-producing effect of sympathomimetic amines. The cat soleus muscle, which is very sensitive to β-adrenoceptor agonists, responds to them by a decrease in the tension and duration of the maximal twitch, and a pronounced decfease in the fusion of incomplete tetanic contractions (Bowman & Zaimis, 1958; and for a review see Bowman & Nott, 1969).

In the experiments described in this paper, the effects of several sympathomimetic amines have been compared on the cat soleus muscle preparation. A procedure for constructing cumulative dose-response curves is described. In comparison with methods in which responses to single doses of the amines are recorded, this method was quick and gave reproducible results.

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Methods.—Cats were anaesthetized by the intraperitoneal injection of a mixture of chloralose (80 mg/kg) and sodium pentobarbitone (6 mg/kg). The trachea was cannulated and blood pressure monitored from a cannulated carotid artery (1 mmHg≡1·333 mbar). The soleus muscle was prepared for recording isometric contractions as described by Bowman & Nott (1970) and the muscle nerve was stimulated once every 10 s at a frequency of 8 Hz for 1 second. In each experiment the resting tension was frequently checked and maintained at a constant level (40–80 g in different experiments). Drugs were injected intravenously through a cannulated brachial vein, either as single doses, or cumulatively. During cumulative administration, each dose was given at the height of the response produced by the previous dose in the series. The injections were continued until maximal response was obtained.

Results.—Figure 1a shows the results of one of four experiments in which cumulative dose-response curves to (−)-isoprenaline were established alternately with responses to single injections of the amine. Similar depressions in submaximal tetanic contractions were obtained with given doses of (−)-isoprenaline by both methods of injection. In these experiments the curve obtained from plotting the results from single injections lay from 0·005 to 0·03 log units to the left of the mean curve obtained from cumulative dose-response curves. This small shift is probably explained by the inactivation of the amine, so that a lower effective concentration of (−)-isoprenaline is present when given cumulatively than when the same total dose is given as a single injection. In any one experiment cumulative dose-response curves for (−)-isoprenaline were highly reproducible when the curves were produced at 30 min intervals (Fig. 1a). In twelve experiments, in which two or more curves for (−)-isoprenaline were established, the mean maximal separation of the curves (±S.E.) taken at 50% of the maximum depression was 0·023 ± 0·004 log units. The responses obtained in different animals were also reproducible. In thirteen cats, the mean cumulative dose (±S.E.) of (−)-isoprenaline required to produce 50% of the maximal depression in 29 cumulative dose response curves was 0·068 ± 0.005 μg/kg, and the mean (±S.E.) maximal depression of the incomplete tetanic con-

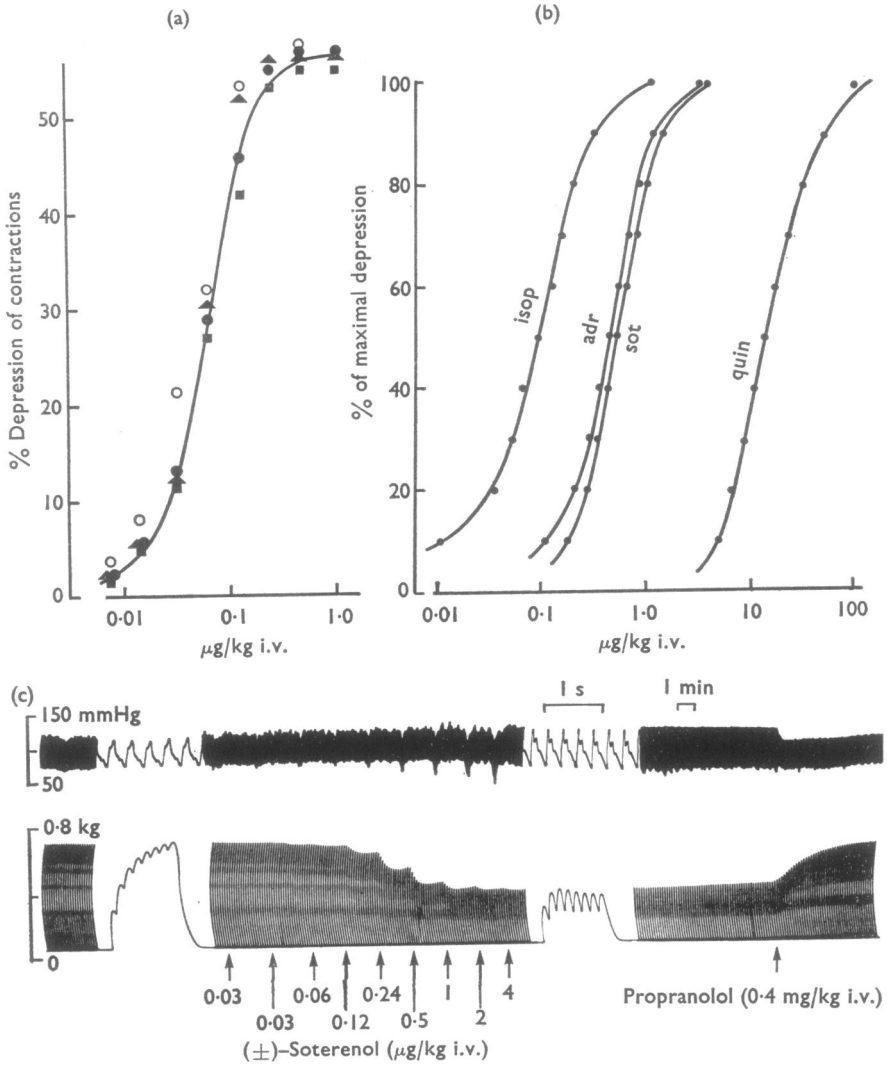

FIG. 1. Depression of submaximal tetanic contractions of the cat soleus muscle by (−)-isoprenaline and other amines. (a), Results from an experiment in which three cumulative dose-response curves to isoprenaline were established in the following order ■, ● and ▲. Responses to single injections of isoprenaline are shown (○). (b), Effects of (−)-isoprenaline (isop), (−)-adrenaline (adr), (±)-soterenol (sot) and (±)-quinterenol (quin) on soleus contractions. Responses are expressed as percentages of the maximal depression produced by (−)-isoprenaline in five experiments with each drug. (c), An experiment to determine the dose-response relationship for soterenol. Contractions of a soleus muscle were evoked by stimulating the motor nerve at a frequency of 8 Hz for 1 s every 10 seconds. The pen recording includes two contractions recorded on fast moving paper. The upper record is of general arterial blood pressure. Propranolol was injected during the prolonged response to soterenol.

tractions, expressed as a percentage of control, was 48·9±1·5%.

Cumulative dose-response curves were also produced using (−)-adrenaline, (±)-soterenol and (±)-quinterenol. The last two drugs were chosen as examples of compounds with a long duration of action as β-adrenoceptor stimulants (Dungan, Cho, Gomoll, Aviado & Lish, 1968; Scriabine, Moore, Iorio, Goldman, McShane & Booher, 1968; Bowman & Nott, 1970). In experiments with these drugs, curves for (−)-isoprenaline were first produced and then the long acting drug was injected

cumulatively. The maximal response to any dose in a series was attained within 45–90 s with (−)-adrenaline and (−)-isoprenaline, 1–2 min with (±)-soterenol, and 15–30 min with (±)-quinterenol. The slow onset of the β-adrenoceptor stimulant activity of (±)-quinterenol has been noted previously by other authors (Scriabine *et. al.*, 1968; Bowman & Nott, 1970). Likewise, the offset of the drug actions differed. Responses returned from maximal to half maximal depression in 3–5 min with (−)-adrenaline and (−)-isoprenaline. In two of five experiments with (±)-soterenol, the mean time to half return of the responses was 40 min, and in the remaining experiments was >1·5 hours. After maximal depression of the contractions had been obtained with (±)-quinterenol there was no significant restoration of the responses within 2 hours.

Figure 1b shows plots of cumulative dose-effect curves for (−)-isoprenaline, (−)-adrenaline, (±)-soterenol and (±)-quinterenol, responses being expressed as a percentage of the maximal response to (−)-isoprenaline in each experiment. Figure 1c shows a trace in which (±)-soterenol was given cumulatively. All four agents produce the same maximal depression of the incomplete tetanic contractions and the dose-response lines are parallel.

Potency ratios were calculated at dose levels required to produce 50% of the maximal depression. The means (±S.E.) of the potency ratios from five comparisons of each drug with (−)-isoprenaline, showed that on a weight basis, (−)-adrenaline, (±)-soterenol and (±)-quinterenol were 5·1±0·5, 5·3±0·7 and 144±43, and on a molar basis 5·5±0·5, 4·1±0·5 and 108±32 times less potent than (−)-isoprenaline in producing depression of incomplete tetanic contractions of the soleus muscle. This potency ratio for (−)-adrenaline is similar to that obtained in experiments where the amines were injected as single doses, in which case, (−)-adrenaline was found to be 5·9±0·6 times less potent than (−)-isoprenaline on a weight basis.

In six experiments the maintained depression of the soleus muscle contractions produced by (±)-soterenol or (±)-quinterenol was reversed by the intravenous injection of the β-adrenoceptor antagonist, propranolol (0·4 mg/kg) (Fig. 1c).

Discussion.—In evaluating sympathomimetic amines for their ability to decrease the fusion of incomplete tetanic contractions of the soleus muscle (Bowman & Nott, 1970; Brittain, *et. al.*, 1970), single doses of the compounds have been injected and relative potencies calculated from dose-response curves. This method suffers from the disadvantage that it is extremely time consuming to have to wait for full recovery before giving the next dose. Furthermore, with the single dose regime, it is impossible to construct dose-response curves to long-acting drugs such as (±)-quinterenol (Bowman & Nott, 1970).

The present experiments show that cumulative drug administration allows reproducible results to be obtained relatively quickly when short acting amines such as (−)-isoprenaline and (−)-adrenaline are used. In addition, full dose-response curves may be obtained with long acting compounds such as (±)-soterenol and (±)-quinterenol.

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