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The guinea-pig isolated vas deferens: a method for increasing sensitivity to drugs

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A preparation of the guinea-pig vas deferens was developed in which it was opened along its length. It was sufficiently sensitive to obtain reproducible dose-response curves to acetylcholine, noradrenaline, histamine, nicotine, 5-hydroxytryptamine and potassium chloride; it was more sensitive than the unopened vas deferens.

THE isolated hypogastric nerve–vas deferens preparation (Huković, 1961) has been widely used for physiological and pharmacological, investigations. The mixed pre- and post-ganglionic nature of the hypogastric nerve and the proximity of the ganglia to the wall of the organ (Sjöstrand, 1962; Ferry, 1963; Ohlin & Strömblad, 1963) led to the development of a method for transmural stimulation to obtain a preparation stimulated through its post-ganglionic nerves (Birmingham & Wilson, 1963). Even when stripped of its mesenteric investment this adrenergically innervated organ is comparatively insensitive to exogenously applied drugs, and has therefore been rarely employed except in conjunction with hypogastric nerve or transmural stimulation (Bentley & Sabine, 1963; Birmingham, 1966). We have been able to modify this preparation, making it sufficiently sensitive for the study of drugs in the absence of electrical stimulation.

Experimental

Vasa deferentia from albino guinea-pigs (250—350 g), killed by cervical dislocation and bled by section of the carotid ateries, were isolated without dissection of the hypogastric nerve; the organs were carefully freed of the mesenteric investment according to the method of Bentley & Sabine (1963). Next, one blade of a pair of fine scissors was inserted into the urethral end of the lumen and the organ cut open longitudinally. The inside surface of the opened vas was carefully scraped free of mucilagenous material. The preparation was placed in a 5 ml organ bath, containing modified Krebs Ringer bicarbonate buffer (Hukovic, 1961), prepared with distilled, demineralized water and aerated with 5% carbon dioxide in oxygen. The temperature was kept at $37 \pm 0{\cdot}5°$. Tension, 200–300 mg, was applied to the organ.

At the beginning of each experiment the preparation was permitted to equilibrate for 1 hr, during which period the bath fluid was exchanged three to four times by bottom drainage. Dose-response curves to acetylcholine, noradrenaline, histamine and potassium chloride were obtained by adding doses every 4 min and washing repeatedly between each application. Doses of 5-hydroxytryptamine (5-HT) and of nicotine were added at 20- and at 90-min intervals, respectively. The agonists were randomly alternated in successive experiments. Between each dose-response curve a washing and resting period of 10 to 20 min was interposed. The maximum contractile responses were recorded as grams of

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tension by a Grass force transducer (FT-03) using a Grass or Sanborn polygraph.

The compounds studied, expressed as salt concentrations per ml of bath fluid, are: Acetylcholine bromide (Eastman Organic Chemicals, Inc.), histamine dihydrochloride (Mann Research Laboratories), (—)-noradrenaline bitartrate (Sterling Winthrop Research Institute), nicotine hydrogen tartrate $2H_2O$ (British Drug Houses), 5-HT creatinine sulphate, H_2O (Mann Research Laboratories) and potassium chloride (reagent grade, Fisher Scientific Co., Inc.). Concentrated stock solutions were made in distilled, demineralized water except that of noradrenaline which was dissolved in 0·1 N HCl; they were frozen between use and stored no longer than 10 days. Appropriate serial dilutions were made with the Krebs buffer. Linear regression analysis of the results was obtained by the method of least squares (Snedecor, 1956), using an Olivetti Underwood Programmer 101, programmed by the manufacturer.

Results

The preparation contracted maximally to all agonists within 1 min except to 5-HT for which 2–3 min were required. Contractions to each agonist declined quickly, even before washing, except to nicotine which caused a contraction lasting up to 1 min, and to 5-HT which sometimes induced long lasting rhythmic waves of contractions. The results are summarized in Fig. 1.

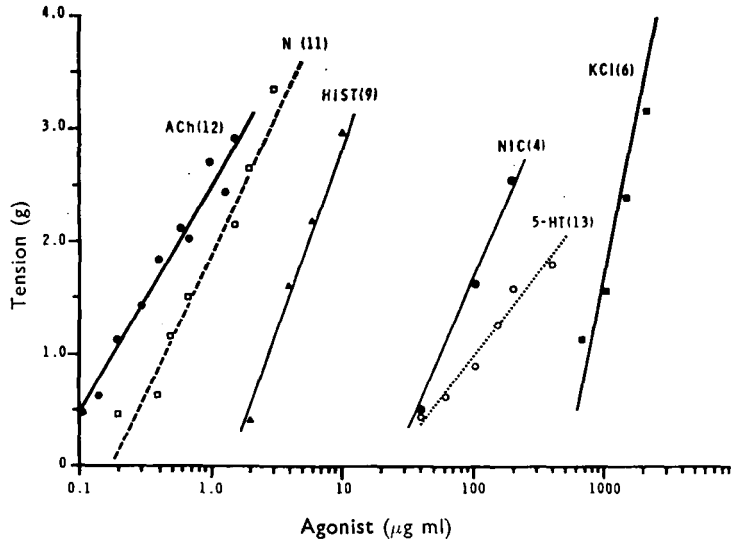

FIG. 1. Dose-response curves with regression lines, obtained by least squares calculations, to acetylcholine (ACh ● slope ± s.e. 2·00 ± 0·13), noradrenaline (N □ 2·50 ± 0·24), histamine (HIST ▲ 3·21 ± 0·26), nicotine (NIC ● 2·88 ± 0·08), 5-HT (5-HT ○ 1·5 ± 0·13) and KCl ■ 5·91 ± 0·46) are depicted. The figures in brackets indicate the number of organs studied for each point shown for each line. The dose-response regression line to nicotine was obtained, using single organs of animals weighing over 300 g.

As can be seen, the steepest slope was obtained with potassium chloride which was significantly greater than that obtained with histamine ($P<0.01$). The dose-response curve to noradrenaline had a slope similar to that to acetylcholine, and was significantly lower than that to histamine ($P<0.001$). Although the curve to 5-HT appears flatter (Fig. 1), the difference in slope when compared with noradrenaline was not significant ($P<0.1$).

The tissue was most sensitive to acetylcholine, which produced contractions at concentrations as low as 0·1 μg/ml. Noradrenaline, at a concentration of 0·2 μg/ml, consistently produced a contraction, whereas it was necessary to use 2μg/ml of histamine and as much as 40μg/ml of nicotine and 5-HT. Potassium chloride at its threshold concentration, 7,500 to 10,000 times that of acetylcholine, produced a very brisk response. If left in contact with the tissue for a longer period, potassium chloride often induced secondary contractions; therefore, it was washed out immediately after a response was observed. All dose-response curves were reproducible, except that not every vas responded equally well to 5-HT and to nicotine. The contractile responses to 5-HT were greater in organs of animals weighing less than 275 g, while nicotine produced greater contractions in organs taken from guinea-pigs weighing more than 300 g. The regression line to nicotine was obtained using organs only of animals weighing over 300 g.

When the vas was stripped but not opened and scraped, much higher concentrations of all agonists had to be applied, and these concentrations often induced spontaneous waves of contractions which persisted even after repeated washing. Under these conditions reproducible dose-response curves could not be obtained.

Discussion

The opened and scraped vas deferens of guinea-pigs can be used to obtain reliable dose-response curves to acetylcholine, noradrenaline, histamine, nicotine, 5-HT and potassium chloride. With the exception of 5-HT which sometimes induced rhythmic contractions, washing of the preparation after agonist administration produced a rapid return to the baseline. These findings are in contrast to those of Ohlin & Strömblad (1963) who showed that noradrenaline and acetylcholine induced spontaneous movements in vasa deferentia, even at 31°. Laporte, Jané & Valdecasas (1966) studied guinea-pig vasa deferentia at 30° and reported that 5-HT did not produce a contractile response. Furthermore, their preparation, which was not stripped, was considerably less sensitive than the stripped and opened organ. They reported an ED50 for acetylcholine of 2 μg/ml and for noradrenaline of 5 μg/ml, while our results showed the opened vas to be approximately 10 times more sensitive. The ED50 of our preparation was 0·3 μg/ml for acetylcholine and 0·6 μg/ml for noradrenaline. The opened vas deferens thus is a reliable and sensitive preparation which permits the study of effects of drugs on this smooth muscle.

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