DEMONSTRATIONS

A modular system of instruments for simple experiments on skeletal muscle using intracellular microelectrode techniques

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The apparatus to be demonstrated forms a basic system used routinely in three laboratories over the last five years. The system consists of a buffer amplifier, constant current generator, current monitor and differentiator. The circuits are essentially derived from those described by Clayton (1971).

The buffer amplifier employs a 748 operational amplifier with a dual field effect transistor (F.E.T.) source follower at the front end. It features input capacitance neutralisation and a facility for checking electrode resistance. The input impedance of the amplifier is of the order of 10^{12} ohms and a typical rise time of 60 μs is obtained when used with a 10 MΩ electrode connected to the amplifier via a 25 cm long low noise input lead.

The constant current generator consists of a high gain amplifier with a dual F.E.T. front end with two input and two feedback resistors connected to form a constant current generator. Additional transistor switching circuitry is employed to drive the generator at the required trigger voltages, enabling it to produce both steady and pulsed currents of up to ± 200 nA.

The timing of the pulse current is obtained by triggering the generator from two output sockets of a Digitimer Constant Voltage Generator.

A capacitance neutralization amplifier similar to the buffer amplifier is also built-in enabling the user to square up the stimulating current pulse and to monitor the condition of the microelectrode. An electrode resistance measuring facility is also employed.

The current monitor is also a high gain amplifier with a dual F.E.T. front end with a single feedback resistor. Connected this way the circuit forms what is commonly known as a current to voltage converter, producing a voltage output directly proportional to the current being monitored.

The differentiator circuit is typical of 'active' differentiating circuits for measuring the rate of rise of action potentials and is meant to be driven from the low output impedance socket of the buffer amplifier. It features an input impedance of 1 MΩ and will give an output of 1 V maximum for rates of rise of either 0–1000 V/sec or 0–10 V/seconds.

The apparatus is constructed as a modular system allowing considerable flexibility in use. It is easily assembled and has a total component cost of approximately £250. A full description of the circuit is available.

Reference

CLAYTON, G.B. (1971). *Operational Amplifiers*. London: Butterworths.

A modified guinea-pig stomach preparation

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Several isolated preparations have been used to investigate the motility of the guinea-pig stomach (for references see Beani, Bianchi & Crema, 1971) but all these preparations were relatively insensitive to relaxant drugs and the relaxations were slow in onset. In the isolated preparation demonstrated, these problems have been overcome by injecting drugs into the vasculature of the stomach, via the coeliac axis.

Female guinea-pigs (250–400 g) were starved for 24 h before being killed by cervical dislocation. The vagal nerves were located and dissected clear of the oesophagus, which was ligated. The coeliac axis was cannulated before the stomach was removed from the animal; the spleen was ligated and removed, ensuring that the blood supply to the greater curvature of the stomach was intact. A bulk ligature was tied around the fat surrounding the hepatic artery and the pancreatic and duodenal artery was ligated. Freshly oxygenated McEwen's solution (4 ml) was slowly injected through the vasculature. The stomach, with the cannula attached, was then removed from the animal and a hole cut in the fundus to wash the lumen clear of chyme. The tube of a modified Trendelenburg apparatus was tied into the fundus, the pylorus was ligated and the preparation set up in a 100 ml isolated organ bath filled with McEwen's solution maintained at 35 ± 1°C, gassed with 95% O_2: 5% CO_2. The tube of the apparatus was connected to a reservoir of McEwen's solution; the level of fluid in the reservoir was initially set 3 cm above the fluid level in the organ bath. Air pressure over the reservoir was measured using an Ether UPI air pressure transducer and a Devices M2 recorder.

Stimulation of the vagal nerves resulted in a contraction of the stomach and a displacement of McEwen's solution from the lumen of the stomach into the reservoir. Hyoscine (0.3 μM) converted the contraction into a relaxation; the increase in volume of the stomach following vagal stimulation (5 Hz for 10 s) was 2.0 ± 0.4% (s.e. mean, $n=13$; resting volume 29 ± 6 ml). In the presence of hyoscine, relaxations were consistent for 30 hours.

Drugs, dissolved in 0.1 ml McEwen's solution, were injected via the coeliac axis and followed by 0.4 ml McEwen's solution. Relaxations induced by (−)-noradrenaline (0.1–10 nmol) and ATP (0.1–10 μmol) were rapid in onset and produced a maximum effect within 10 seconds. The relaxations resembled the responses to vagal stimulation the presence of hyoscine. The drug-induced relaxations were not tachyphylactic and were not antagonized by tetrodotoxin (0.3 μM). The preparation was more than 80 fold more sensitive to noradrenaline and more than 30 fold more sensitive to ATP when the drugs were injected via the coeliac axis than when added to the McEwen's solution in the organ bath.

Reference

BEANI, L., BIANCHI, C. & CREMA, A. (1971). Vagal non-adrenergic inhibition of guinea-pig stomach. *J. Physiol., Lond.*, **217**, 259–279.

Regional perfusion of the airways of guinea-pig lung: a selective action of histamine on the smooth muscle of the small airways

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The direct actions of drugs on the smooth muscle of lung have usually been investigated using muscle of the larger airways (Castillo & de Beer, 1947; Coleman & Farmer, 1970) despite evidence from experiments *in vivo* that the principal site of action is the fine peripheral airways (Drazen & Austen, 1974). The actions of histamine and acetylcholine on the perfused airways of guinea-pig lungs have been investigated using modifications of the preparation of Sollman & Von Oettingen (1928).

Lungs were removed from guinea-pigs (350–650 g) and washed free of blood with Krebs solution (NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; MgCl 1.2; $NaHCO_3$, 25.0; NaH_2PO_4 1.2; glucose 11.5 mM) via a cannula in the pulmonary artery. Whole lungs were perfused via a polythene cannula tied into the trachea, the tip lying about one-third of the distance from the larynx to the bifurcation of the major bronchi. The fluid escaped from fine scarifications on the surface of the lobes. Half lungs were cannulated through the lower trachea and the cannula tied into the bronchus so that the tip lay 2–4 mm beyond the bifurcation. Single lobes were perfused via a fine cannula introduced through the bonchus such that the tip lay in the centre of the lobe; all other lobes were tied off and removed. Preparations were suspended in a constant temperature chamber and perfused with Krebs solution gassed with 5% CO_2 in O_2 at 5, 2.5 and 2 ml/min respectively. Perfusion pressure was measured by a transducer attached to a side arm above the cannula. Agonists were injected into the perfusion fluid in volumes of 0.1 ml through a thick-walled elastic tubing between the side-arm and the cannula.

Acetylcholine (100 ng–200 μg) and histamine (10 ng–100 μg) caused dose-dependent increases in perfusion pressure in all preparations. The order of sensitivity to both agonists was left lower lobe > right lower lobe and upper lobes ≥ half lungs > whole lungs (ED_{50} for histamine = 25 : 141 : 141 : 4000 ng respectively and for acetylcholine = 0.63 : 3 : 14 : 18 μg respectively). Also the maximum pressure attained with left lower lobes (mean ± s.e. = 155 ± 18 mm Hg; $n=14$) was greater than that attained with other preparations (66 ± 10 mmHg; $n=20$).

The greater sensitivity of perfused lobes compared with whole lungs to histamine (ratio ED_{50} $s=16$) against acetylcholine (ratio ED_{50} $s=2.9$) suggests that histamine has a relatively greater effect on smaller airways. In addition, the perfused single lobe provides a very sensitive simple preparation for the study of drugs on the smooth muscle of small airways.

References

CASTILLO, J.C. & de BEER, E.J. (1947). The tracheal chain. *J. Pharmac. exp. Ther.*, **90**, 104–109.

COLEMAN, R.A. & FARMER, J.B. (1970). A new preparation of the isolated intact trachea of the guinea-pig. *J. Pharm. Pharmac.*, **22**, 46–50.

DRAZEN, J.M. & AUSTEN, K.F. (1974). Effects of intravenous administration of slow reacting substance of anaphylaxis, histamine, bradykinin and PGF on pulmonary mechanics in the guinea pig. *J. clin. Invest.*, **53**, 1679–1685.

SOLLMAN, T. & von OETTINGEN, W.F. (1928). Bronchial perfusion of isolated lung as a method for studying pharmacological reactions of bronchiolar muscle. *Proc. Soc. exp. Biol.*, **25**, 692–695.