1 Introduction

We are investigating how the dynamics of signal transmission in endocrine systems relates to the mechanisms involved. Evidence is accumulating to suggest that information transfer in a number of endocrine systems depends on rapid modulation of hormone levels in pulses. This kind of signalling mechanism should be more efficient than one depending on merely the average level of a hormone in the bloodstream (McIntosh and McIntosh, 1980), as was formerly believed to be the case. In order to propose mechanisms there is a need to characterise in detail the effects of pulsed signals on an appropriate endocrine target.

Our particular interest is in the hypothalamic-pituitary-gonadal axis and our current experimental system consists of suspensions of sheep pituitary cells free or attached to microbeads, which are stimulated to release the gonadotrophins luteinising hormone LH and follicle stimulating hormone FSH by applying transient pulses of the hypothalamic peptide gonadotrophin releasing hormone GnRH. Because we are interested in determining exactly which characteristics of the releasing hormone pulse are important in controlling the secretion of gonadotrophins from pituitary cells we needed a way of delivering several different patterns of GnRH stimulatory signals simultaneously to aliquots of a single preparation of cells automatically. The advantage of simultaneous stimulation of identical samples is that any time-dependent changes in responsiveness of the biological material should be the same for all samples; differences in response between samples can then be attributed to differences in the stimulation patterns applied.

We describe here a microcomputer-controlled device which is flexible enough to meet these requirements and yet simple and inexpensive to construct from readily available components with only minimal workshop facilities.

2 Design and construction of the apparatus

Instructions describing the desired pattern of stimulation are entered into the memory of the microcomputer by a series of simple commands at the beginning of the experiment. These instructions, together with a program stored on a cassette, cause the computer to activate solenoid valves at required times so as to introduce stimulatory hormone into the medium perifusing the cells. The medium is passed over the cells continuously by a 15-channel peristaltic pump and collected in a fraction collector controlled by the microcomputer and modified to collect up to 15 fractions simultaneously. The fractions are later assayed for hormone release from the cells and the relationship between fraction number and time of stimulation is found conveniently from a record of time, fraction change and stimulation pattern printed by the microcomputer.

A schematic diagram of the complete apparatus is shown in Fig. 1. Components already available or readily acquired were the cassette recorder, the fraction collector (model IF 1301, Electrothermal Engineering Ltd., London E7 9QN, UK) and the multichannel pump (Technicon Instruments Corp., Tarrytown, NY 10591, USA or Chemlab Instruments Ltd., Hornchurch, Essex RM11 3XJ, UK).
2.1 Microcomputer

The microcomputer must have some form of clock and at least 16 output channels. There should be a convenient keyboard for program entry, and facilities must exist for recording and reading memory from and to a cassette recorder. A suitable instrument is the AIM 65 (Rockwell International Corp., Microelectronic Devices, Anaheim, CA 92803, USA)—neither the extended assembler nor other options are required. The AIM 65 also has a small printer attached which provides a convenient record of each experiment. A power supply (+5 V, 2 A regulated DC and +24 V, 2.5 A DC) is required.

2.2 Interface

The logic signals appearing on the output terminals of the microcomputer must be converted to electric currents capable of operating the solenoid valves and also a relay activating the fraction collector. For this purpose an interface was constructed (Fig. 2). Logic signals from the output of the microcomputer were applied to the appropriate solenoid through a NOR gate (IC 0) which allowed the solenoids to be operated manually, either individually or all together), an open-collector noninverting buffer serving as a level translator and a switching transistor T 1. On activation each switching transistor conducted a current to the appropriate solenoid. Each of the 15 solenoids required about 300 mA to close but only about 80 mA to hold once closed; thus, to minimise the current drawn from the power supply, capacitor C 1 (2200 µF, 25 V) was included to provide the initial current transient necessary to close each solenoid. Once closed, the current flowing through R 4 and T 1 was sufficient to both hold the solenoid closed and recharge C 1.

The interface components were assembled on boards and mounted in a metal box with 16-way sockets for connecting cables from the microcomputer and the solenoids/fraction collector relay. The interface logic required +5 V, 0.5 A regulated DC and the solenoids about +18 V, 2 A DC.

2.3 Solenoid valves

Practical constraints demanded that the valves, which when activated switched the flow of nutrient medium perusing the cells (0.02-0.2 ml min⁻¹) to a medium containing stimulating hormone, should have negligible dead volume so as to take full advantage of the small internal diameter (0.2 mm) of the connecting tubing (Tygon). Equally important was that the valves should be mechanically simple, inexpensive and easy to construct.

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