DEMONSTRATIONS

A microcomputer-based signal acquisition system suitable for use in the pharmacological and physiological laboratory

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A digital signal acquisition package has been developed for use in conjunction with the Commodore PET-2001 microcomputer. It features a multichannel analog-to-digital converter (National Semiconductors ADC 0817) and a single-chip digital-to-analog converter (Ferranti ZN 425 or equivalent). These devices are interfaced to the memory bus of the microcomputer using MOS 6522 Versatile Interface Adaptors, which also contain useful timing registers.

Although data handling can be performed using BASIC programs (Baillie & Smith, 1979), this can be slow, and greater efficiency and speed can be achieved using machine code subroutines for data capture and display. A block of memory is reserved as a data buffer, and a simple subroutine written in 6502 machine code controls the sequential digitization and storage of 256 (or 512 or 1024 etc) 8-bit data values in the buffer. The contents of this array of 'captured' data can then be sequentially accessed for display via an analog-to-digical converter and oscilloscope or pen-recorder, or can be stored on mass storage media such as magnetic tape or disc.

Facilities also exist for the internal generation of a wide variety of mathematical functions which can be displayed on the oscilloscope or written to a pen recorder.

The system offers many of the facilities available on transient recorders, e.g. capture of high-speed transients and their subsequent play-back at lower speed, but also allows the programmer to perform more sophisticated analysis such as signal averaging, measurement of maxima and minima or duration of waveforms, and even procedures such as spectral analysis could be performed.

The package could readily be adapted for use on other microcomputer systems. Current applications include the recording of transients in electrophysiology and the acquisition and analysis of data from respiratory experiments.

The approximate cost of external components is £50.

Reference

BAILLIE, P. & SMITH, I.C.H. (1979). Real-time computing in the teaching laboratory—an analysis of heart-rate transients. *J. Physiol. proceedings* (In press).

The following demonstrations were given at the Aston meeting of the British Pharmacological Society, 4th–6th April, 1979

The binding of [^{3}H]-noradrenaline and [^{14}C]-propranolol to synaptosomal fractions of rat brain homogenates

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Propranolol has been shown to inhibit both uptake and tyramine-induced release of noradrenaline in the periphery (Foo, Jowett & Stafford, 1968). More recently, it has been suggested that propranolol itself may be taken up into peripheral adrenergic neurones, stored, and released along with noradrenaline (Daniell, Walle, Gaffney & Webb, 1978), although there is conflicting evidence on this point (Lewis, 1977). Penetration of propranolol into the brain after systemic administration has been demonstrated by a number of workers (Laverty & Taylor, 1968; Hayes & Cooper, 1971; Street, Hemsworth, Roach & Day, 1979), but the action of this drug upon central adrenergic neurones has not been studied in detail. The present work was undertaken to investigate the possible inhibitory effects of propranolol upon noradrenaline uptake into synaptosomes prepared from rat brain homogenates, and further, to investigate

the possibility that propranolol itself may be taken up into synaptosomes by the same mechanism responsible for noradrenaline uptake.

Whole brains were removed from male Wistar rats (250–350 g) and crude synaptosomes (P_2 fraction) were prepared by the method of Whittaker, Michaelson & Kirkland (1964). After separation, the P_2 pellet was resuspended in ice-cold Krebs-Ringer phosphate buffer, and this suspension was used for the incubations. Incubations were performed at 37°C for 7 min, and the incubation medium contained 0.1 ml of the synaptosome suspension in a total volume of 2.0 ml Krebs, incorporating (−)-[^{3}H]-noradrenaline hydrochloride or (±)-[^{14}C]-propranolol hydrochloride. Glucose (10 mM), ascorbic acid (0.2 mg/ml), EDTA (0.1 mg/ml) and nialamide (1.25×10^{-5} M) were also present. Incubations were terminated by cooling and centrifugation, and the synaptosomes were then washed, solubilised and assayed for total radioactivity by liquid scintillation spectrometry.

[^{3}H]-noradrenaline was taken up into synaptosomes by a saturable high-affinity uptake process, with a K_m of 0.28 μM and a V_{max} of 5.7 pmoles NA mg protein^{-1} minute^{-1}. At a substrate concentration of 1.0×10^{-7} M, uptake was found to be Na^+-dependent and temperature-sensitive. At the same substrate concentration, cocaine (1.6×10^{-7} M), desipramine (1.7×10^{-7} M), ouabain (2.8×10^{-6} M), propranolol (1.9×10^{-5} M), oxprenolol (2.8×10^{-5} M) and metoprolol (8.8×10^{-5} M) were all inhibitors of uptake, and the IC_{50} values for these inhibitors are shown in parentheses. In contrast, the uptake of [^{14}C]-propranolol, at a substrate concentration of 8.5×10^{-7} M, was not affected by extracellular Na^+ concentration, or by cocaine, desipramine or ouabain at the previously mentioned IC_{50} concentrations. Propranolol uptake was reduced by approximately 30% at 4°C, but this may possibly be explained by the temperature-dependent partitioning of this drug (Street, 1979).

In these experiments, propranolol was an effective inhibitor of synaptosomal noradrenaline uptake, but did not itself appear to be taken up into synaptosomes by the same high-affinity process responsible for the uptake of noradrenaline. It is suggested that the incorporation of propranolol into synaptosomal fractions of rat brain homogenates is largely a function of its lipophilicity.

References

DANIELL, H.B., WALLE, T., GAFFNEY, T.E. & WEBB, J.G. (1978). Stimulation induced release of propranolol and norepinephrine from adrenergic neurons. Proceedings of the *VIIth International Congress of Pharmacology, Paris.* Abstract No. 2416. Pergamon, Oxford.

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The neuromuscular blocking action of some cyclic analogues of choline

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In this study four analogues of hemicholinium-3 (HC-3) have been synthesised, where the interatomic distance between the two morpholinium rings is increased by the insertion of methylene groups, $(CH_2)_n$ where $n = 1$ to 4, between the two phenyl rings. The compound where $n = 0$ is HC-3 itself.

The neuromuscular blocking action of these compounds has been investigated using the rat phrenic nerve-hemidiaphragm preparation (Bulbring, 1946). All the analogues gave a prejunctional block which was reversed by choline (0.1 μm/ml).

A partially purified extract of choline acetyltransferase (ChAC) was obtained from rat brain and incubated at 37°C with [^{14}C]-acetyl CoA and either choline or one of the analogues (20 mM). The amount of acetylation was determined in each case.

A similar incubation system was used to measure