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COMPUTER-SIMULATION OF CARDIOVASCULAR RESPONSES FROM IN VIVO PREPARATIONS

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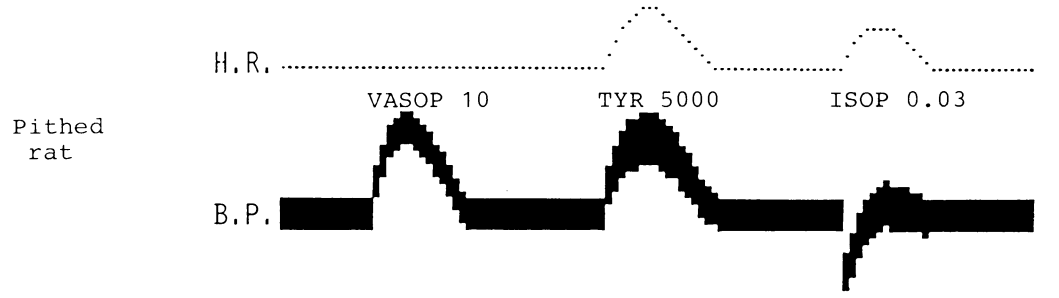
Practical classes in pharmacology often have 3 objectives; 1) to provide 'hands-on' experience to interest the student & exercise laboratory skills, 2) to teach & practise correct experimental design, & 3) to illustrate aspects of pharmacology dealt with in theory classes. The first of these is essential & may involve the use of animals but 2 and 3 can be achieved by other means.

A computer program will be demonstrated which provides a record simulating the trace obtained during experiments involving responses of the cardiovascular system to drugs; anaesthetised (normal or reserpinised) or pithed preparations can be used. Agonists (angiotensin, vasopressin, ADR, NA, ISOP, PE, B-HT920, tyramine, histamine, ACh, DMPP) and blockers (atropine, mepyramine, ranitidine, neostigmine, phenoxybenzamine, prazosin, yohimbine, propranolol, atenolol, nifedipine, cocaine, hexamethonium) are available & vagal & sympathetic cardiac nerves can be stimulated.

The dose & sequence of administration of drugs is entirely in the hands of the user. Once administered, the effects of blockers wear off at a rate corresponding to their t-half & overdose with agonists or blockers will kill the preparation. Tachyphylaxis is seen with some agonists, responses are subject to biological variation & are influenced by the presence of cardiovascular compensatory reflexes as appropriate. Responses of heart rate and blood pressure (systolic and diastolic) to drugs take about 10 seconds to be printed on a standard printer (see below) but occupy about 3 minutes of 'preparation time' - preparations deteriorate slowly and are liable to die unexpectedly after about 6 hours of preparation time have elapsed. 'Unknowns' can be provided by the program & the student asked to use the preparations to characterise these drugs.



The program provides an inexpensive way of illustrating drug effects on the cardiovascular system and is especially good at exercising & teaching experimental design so students can make maximum use of their 'hands-on' experience of whole animal preparations.

ADVANCED IMAGE ANALYSIS OF RECEPTOR BINDING AUTORADIOGRAPHS

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Tritium film autoradiography provides a powerful technique for visualising, using ^{3}H- or ^{125}I-labelled ligands, the discrete distribution of specific receptor binding sites (Palacios et al, 1981; Penney et al, 1981). Autoradiographs are generated by co-exposure of cryostat sections, labelled with a radioactive receptor ligand, and calibrated radioactive standards. Specific receptor binding may be defined in the presence and absence of an excess of a displacing ligand. Quantitative estimates of both the density and affinity of the binding sites can then be obtained by computer-assisted image analysis of the autoradiographs (Altar et al, 1984; Tayrien and Loy, 1984).

A program in Pascal has been developed for an image analysis system (Magiscan 2A, Joyce-Loebl) which allows optical density calibration and linear transformation of a video camera autoradiographic image and also permits superimposition and subtraction of such transformed images. This latter operation enables the computed distribution of specific ligand binding sites to be displayed and thus facilitates study of the regional distribution of receptor sub-types.

The autoradiographic images are viewed under even, transmitted illumination using a high quality video camera fitted with a zoom lens. The video signal is digitised, converted to 6 bit grey values, fed into an image store (1024x1024x8 bits) and processed by a microprogrammable 16-bit central processor. The image is displayed on a high resolution black and white monitor and an RGB colour monitor. The aperture control on the zoom lens is adjusted to give the maximum illumination without saturation (indicated by red on the RGB monitor). The grey level image is converted to an image of the optical density, within the limits of the dynamic range of the video camera, by integrating 4 images of the illuminated area in the presence and absence of the autoradiograph and subtracting the logarithmically transformed grey level images. This image, at 8-bit resolution, is used for optical density measurements and is displayed as a negative image at 6-bit resolution to allow text and binary overlays. The optical density values of the radioactive standards (for ^{3}H-labelled ligands, Microscales, Amersham), after subtraction of the background density of the autoradiographic film, are measured by sampling either a circular or rectangular area which is defined using a light pen. The best fit curve is computed by polynomial regression analysis and the correlation coefficients together with the data points and calibration curve are displayed. Areas for quantification on optical density images of the autoradiographs are defined using a light pen, after subtraction of the background film density. An optical density frequency histogram as well as the minimum and maximum bin values and their frequencies and the mean value are computed.

Superimposition of a stored and live image can be performed either by outlining key features, alternating between the stored and live images, or by edge enhancement of the images to produce a reference outline. Pseudo-colour images can also be displayed together with a dose/colour calibration to facilitate the perception of regional variations in grey levels.

Altar CA et al. (1984) J Neurosci. Methods, 10, 173-188.
Palacios JM et al. (1981) Neurosci. Letts., 24, 111-116.
Penney JB et al (1981) Science, 214, 1036-1038.
Tayrien MW and Loy R (1984) Brain Res. Bull., 13, 743-750.