A new approach for a pyrogen test

ROSE E. HARTLEY, A.C.C. TSEUNG[1], & B.K-C. YUEN[1]

National Institute for Biological Standards and Control, Hampstead, and Department of Chemistry, City University, London[1].

Using a sensitive oxygen sensor cell a correlation has been found between a rise in body temperature and increased oxygen consumption in experimental animals injected with pyrogenic substances.

Oxygen consumption was measured with individual rabbits or groups of six mice in a controlled environment and air flow and the oxygen concentration in the outlet was measured continuously with a sensitive oxygen sensor developed in the City University, London.

In rabbits injected intravenously with pyrogenic substances, measurement of body temperature with a rectal probe and oxygen consumption were carried out simultaneously. Results with the two methods correlated well:

$$V_T R = V_T Xss + Q \quad (1)$$

$$\Delta t = \frac{V_T(Xss - X^1SS)}{PQ} \quad (2)$$

where V_T = air flow rate in cc/min, R = % oxygen in the inlet, Q = oxygen consumed by the animal before injection in cc/min and P = % increase in metabolic rate/°C rise in body temperature = 13%.

Similar oxygen consumption studies were carried out on groups of six mice prewarmed for 1 h at 37°C. When the IRP was injected intravenously into mice, the mean rise of calculated body temperature with 15 μg/kg (six experiments) was 0.91°C (±0.08) and with 1.5 μg (five experiments) was 0.53°C (±0.07).

Thus results of preliminary experiments suggest that measurement of the metabolic response of mice to a pyrogen using a non-contract method might form the basis of an alternative pyrogen test.

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Pyrogen	Dose (μg/kg)	Rise in body temperature, Δt (°C) Calculated from oxygen consumption	Rectal probe
Shigella	0.5	0.64	0.71
endotoxin	2.4	0.52	0.50
	11.5	0.88	0.86
IRP	0.025	0.83	0.82
		0.86	0.85
	2.5	1.88	1.75
		0.68	0.63

IRP = International Pyrogen Reference Preparation (Shigella dysenteriae).

A technique for the investigation of the effects of drugs on hearing

DAVID MENDEL

Rayne Institute, St. Thomas' Hospital, S.E.1.

The compound action potential of the auditory nerve and mid-brain nuclei in response to sound can be recorded from surface electrodes applied to the ear and the vertex of the head. These responses, known as the electrococochleogram (E.C.O.G.) were described by Sohmer & Feinmesser (1967) and have been used in man and animals to investigate hearing.

We have found, as did Lev & Sohmer (1972), five waves in these recordings. They showed that the first wave is the surface manifestation of the cochlear nerve action potential. The second wave comes from the cochlear nucleus, the third from the superior olivary complex and the fourth and fifth from the inferior colliculus. Within limits, the louder the sound the larger the amplitude and the shorter the latency of each wave. Both tend to saturate.

The presence, amplitude and latency of each wave in response to incremental increases in sound intensity has been used in man to investigate function. In animals, anaesthesia has always been used and this may alter the responses.

In the technique described here, fully conscious and unsedated guinea-pigs were lightly restrained in a perspex box inside an acoustic chamber. Skin electrodes were applied to the tragus and the vertex of the head. The pinna was earthed. Two thousand and forty-eight unfiltered clicks, having maximum energy in the 4 kHz range, were played to the animal from a TDH 39 earphone placed at a fixed distance in front of the head at a rate of 10 clicks/s, and at an intensity of 50 dB H.L.

The responses were recorded on a Medelec Mark 2 electro-cochleography apparatus, using filtration (0.25–5 kHz) common mode rejection and signal averaging to extract the signal from electrical noise.

In these circumstances, the responses to a click of fixed intensity are repeatable and stable over a period of 2 to 3 h and are sensitive to the administration of drugs which modify auditory function, either by physiological mechanisms or through ototoxicity.

This model has been used to investigate the efferent control of the ear using drugs which act upon cholinergic neurones.

The results of injecting atropine sulphate (250 μg/kg) depend on the initial values. When these are small and early, the tracings increase in amplitude as if the sound were some 30 to 50 db louder (a doubling of amplitude). The latency increases by up to 0.3 ms as if the sound were some 30 to 50 db quieter. When the responses are initially large and late, atropine has little effect.

Following the administration of physostigmine (0.2 mg/kg), amplitude decreases and latency increases. The effect of the administration of these drugs depends on the initial state of the system. These results support the view that acetyl choline is the efferent transmitter.

References

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A behavioural test applied in an acute toxicity screen

A.P. SILVERMAN

Department of Pharmacology, Materia Medica, and Therapeutics, University of Manchester, Manchester M13 9PT.

Behavioural toxicology will be most useful if it gives early warning of effects found later by conventional methods or finds effects at lower doses. Some years ago (Silverman, 1973) I briefly reported an 'exploration-thirst' test which seems informative and simple enough to investigate as a screen.

A rat explores a new environment. This is usually measured as motor activity but also implies curiosity, and it can also compete with other needs. Rats are deprived of water overnight and placed singly in an unfamiliar cage. Motor activity is scored as pauses/min when the rat stops walking to rear or sniff. The time is recorded (in s) until the rat finds water and begins to drink, if necessary distinguishing between the first lick and the first drink of at least 10 s: the interruption of drinking implies a need for more information about the cage. The rat is returned to the home cage after 5 min, offered water, and the procedure repeated next day.

As a first study of the method's usefulness in practice, it was added to the routine acute intraperitoneal toxicity test on 20 consecutive compounds. Survivors 6 h after injection were deprived of water unless they were obviously ill, and behaviour was observed at 24 and 48 hours.

Sixteen of the 20 compounds showed significant behavioural effects, in 11 at the lowest dose tested and in 12 where no effect was discovered clinically or at post-mortem. In 2 of the cases without behavioural effect, rats had recovered from obvious dyspnoea or convulsions.

Not surprisingly, a near-lethal dose often causes non-specific 'ILLS' (Increased Latency to drinking with Locomotion Slow). Effects on 1 day only indicated recovery or delayed onset. In 7 cases there was post-mortem evidence of peritonitis, at least at higher doses.

The use of two independent measures allows a more specific effect (presumably central) to be distinguished. This was not demonstrated in the present series, but in a subacute inhalation toxicity study, exposed rats walked twice as fast as controls without changing the latency to drinking. Rats exposed to trichloroethylene vapour (100–1000 ppm) drank earlier than controls while walking at the same rate (Silverman & Williams, 1975). Observation suggests other possible CNS causes of non-specific formal