205P THE EFFECT OF Δ^9-TETRAHYDROCANNABINOID AND SR 141716 ON THE APPETITE OF RATS

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Δ9-Tetrahydrocannabinol (Δ9-THC) has been reported to posses inconsistent appetite modulating effects. Studies in humans have reported that cannabis has an enhancing effect on the sensory appeal of food as well as increasing appetite. However in animals there are many reports of a decrease in food intake following Δ9-THC. The increase in appetite in humans has been referred to as the 'munchies', and has been used clinically to stimulate appetite in patients receiving cancer chemotherapy. CB_1 receptors have been reported to be present in several hypothalamic nuclei and are suggested to be involved in appetite modulation. However, some aspects of the Δ9-THC-induced effect on appetite are still not well understood. There have been limited studies to date that specifically have considered the effect of Δ9-THC on appetite and the role of CB1 receptors. In view of this, we investigated the effect of Δ9-THC on consummatory behaviour. The CB_1 antagonist SR141716 (N-piperidine-5-(4-chlorphenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (Compton *et al.*, 1996), was employed to determine the involvement of CB_1 receptors.

Male albino Glaxo-Wistar rats (n=60, 200-350 g) were kept in conditions of constant room temperature (22 °C) and controlled lighting with a 12h light/dark cycle. The animals were adapted to a restricted availability of food paradigm with food being available for 6h (07.00-13.00) commencing 2h after lights on. After eating had stabilised (day 9) animals had a catheter implanted into the external jugular vein (metho-hexitone (18 mg/kg i.p.)/amylobarbitone (30mg/kg i.p.) anaesthesia) in order to allow intravenous (i.v.) drug administration. After two days recovery drug testing occurred. All animals were randomly assigned to receive either vehicle (PVP), 1 ml/kg intraperitonial (i.p.), Δ9-THC (i.v.), 2.5 mg/kg SR 141716 (i.p.) alone or SR 141716 followed 30 min later by Δ9-THC. Comparison of the hourly food intake of groups of drug-treated rats versus their respective control group was performed using a One Way Analysis of Variance and the Student-Newman-Kuels multi-comparison test. Animals treated with Δ9-THC (i.v.) showed a dose dependant inhibition of standard rat food intake, with doses greater than 1 mg/kg Δ9-THC eating significantly less than the control group for the last 5 hours after administration (50% of control intake at 1300h, $p<0.05$, n=5). When administered 30 minutes post access to food, SR 141716 alone significantly decreased food intake after the first hour of feeding (47% of control intake at 1300 h, $p<0.05$, n=5). SR 141716 did not modify the effect of 0.1 and 0.2 mg/kg Δ9-THC except after 4 h of access to food rats pretreated SR†141716 prior to 0.2 mg/kg (9-THC ($p<0.05$, n=5).

The results of the present investigation demonstrate that Δ9-THC produces a dose-dependent inhibition of food intake of partially satiated animals. The inhibition of food intake within the first hour of feeding induced by SR 141716 is noteworthy. Although SR 141716 has previously been described as being devoid of any 'intrinsic' activity, it appears to have activity in our experiments. The time of administration of SR 141716 may be an important factor with respect to its effect on appetite. Alternatively, SR 141716 may block an endogenous cannabinoid system, causing the resultant decrease in food intake. This would assume that an endogenous cannabinoid system stimulates appetite, which is contrary to the effect of the doses of the exogenous cannabinoid used here. In summary, these results suggest that SR 141716 may be acting by blocking an endogenous system (as a CB1 antagonist) as well as antagonising the Δ9-THC-induced effects.

Compton, *et al.*, 1996, J Pharmacol Exp Ther, 277, 586-94.

206P MULTICHANNEL EXTRACELLULAR RECORDINGS FROM A HIPPOCAMPAL NEURONAL NETWORK CULTURE: A DEMONSTRATION

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The use of a Multichannel Acquisition Processor system (MAP, Plexon Inc.) allows large scale real-time neuronal waveform recording and spike sorting from multichannel microelectrode devices. These include microwire bundles, solid-state microprobe arrays or multi-microelectrode culture plates (MMEPs). These technologies provide a powerful tool for studying the physiology and pharmacology of excitable tissues, including neuronal ensembles *in vivo* (Nicolelis *et al.*, 1998), *in vitro* (Gross & Schwalm, 1994) or cardiac myocytes (unpublished data). We will demonstrate its use in recording from hippocampal neuronal networks in culture and its application to studies of epilepsy.

Primary dissociated Wistar rat hippocampal neurones from E18 fetuses were cultured directly onto planar 64-channel MMEPs (Gross & Schwalm, 1994). For recording, the MMEP is connected via two 32-channel amplifiers to signal input boards which provide programmable gain, filtering and analogue-to-digital conversion. A/D conversion is performed by simultaneously sampling 12-bit converters at 40KHz per channel. Signals are then routed to multiple digital signal processor boards (Motorola 56002 DSPs running at 40MHz) for computer-controlled spike waveform capture and sorting. The control software, *RASPUTIN (Real-time Acquisition Systems Programs for Unit Timing in Neuroscience,* Plexon Inc.), for the MAP is implemented in a Server/Client architecture running on a host Pentium PC under Microsoft Windows NT4. During data collection the Server runs a series of on-line clients, with *Sort Client* allowing automatic spike discrimination on all channels with the capability to isolate up to four single-units per microelectrode, principal component analysis of waveforms and recording external events (e.g. drug applications). Additional on-line clients include *NeuroEXplorer* (NEX) and *Graphical Activity Client* which enable on-line data analyses (e.g. integrated firing rate, burst rate, inter-spike interval histograms and cross-correlation analysis) simultaneously with spike capture. On-line data from the Server can also be sent to any other PC either via a local network via *Network Client* and *Data Replicator.* Off-line, data can be manipulated in a number of ways with the spike waveforms re-sorted or the sorted waveforms analysed within NEX, which provides multiple spike-train data analysis package with a very rich set of analysis options and functions. It also provides an open analysis environment to interact with MATLAB, Excel and other mathematical and statistical packages.

In addition to electrophysiological studies of neuronal networks, the system has potential applications in high throughput screens for drug assessment and as a cell-based biosensor (Gross *et al.*, 1997).

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Gross GW and Schwalm FU (1994) *J. Neurosci. Methods* **25**, 73-85.
Nicolelis MAL, Stambaugh CR, Brisben A and Laubach M (1998) In *Methods for neural ensemble recordings*, Eds. Nicolelis MAL, CRC Press, p121-156.

EJ & DMS are supported by BBSRC and GlaxoWellcome

207P A COMPUTER SIMULATION OF THE CAT NICTITATING MEMBRANE PREPARATION FOR UNDERGRADUATE PHARMACOLOGY STUDENTS

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Computer-assisted learning (CAL) programs can support many aspects of teaching and learning in undergraduate pharmacology courses and many examples of these have been demonstrated to the Society in recent years. Laboratory-based practicals which use live animals or animal tissue have long been used to provide a basis for learning by investigation, teaching experimental design and teaching and practicing a wide range of laboratory and research skills. Many universities have been forced to reduce the amount of laboratory work in their curricula and some have turned to computer simulations to provide a 'dry-lab' experience which fulfils some but not all of the objectives of the animal experiments. Simulations may be particularly appropriate where the animal experiment is costly to perform, or requires a high level of technical expertise. One such experiment is the *in vivo* superior cervical ganglion – nictitating membrane preparation of the anaesthetised cat and we present here a computer simulation of experiments that may be performed on this preparation to teach the basic pharmacology of transmission at autonomic ganglia and sympathetically innervated smooth muscle.

The program was written using Macromedia Director version 6.5 for IBM compatible PCs running Windows (minimum specification: PC 486 running Windows 3.1 or better). It has several sections accessible form a menu: *Introduction*: provides information about the program and it's curricula context; *Tutorial:* presents diagrammatic representations of the effector junction and the superior cervical ganglion. This section of the program uses animated sequences to demonstrate the stages of transmission at both the synapse and neuroeffector junction and highlights possible sites of action of drugs; *Methods:* describes the preparation, protocols for nerve stimulation and administration of drugs to the superior cervical ganglion and the nictitating membrane, and the method of recording contractions of the nictitating membrane. *Experiments:* allows students to perform simulated experiments on the preparation and provides recordings of the force of contraction of both ipsilateral and contralateral nictitating membranes which are displayed on a screen designed to emulate a chart recorder. A sub-menu gives students some control over experimental parameters (they can choose: 1. to administer an agent from a list: saline (vehicle control), acetylcholine, noradrenaline, atropine, phentolamine, propranolol, isoprenaline, hexamethonium, physostigmine, nicotine (low and high dose), tyramine, an unknown (which is randomly selected from the list above when the program is run); 2. the site of administration; 3. whether to electrically stimulate preganglionic nerves (half-maximal stimulation). Although it is envisaged that the tutor will develop a set of tasks for students to address when using the simulation which will meet their own teaching objectives, this section does also includes some suggested tasks to aid independent use of the program.

208P THE CARDIOVASCULAR ACTIONS OF CANNABINOIDS: MORE QUESTIONS THAN ANSWERS

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Endogenous cannabinoids (endocannabinoids), which were first identified in the central nervous system, exert cardiovascular actions. In this respect the prototypic endocannabinoid, derived from arachidonic acid, anandamide, is a vasorelaxant, especially in the resistance vasculature. The mechanism(s) by which this occurs has yet to be fully defined. To date, cannabinoids have been proposed to act via the release of endothelial autacoids (NO and prostanoids), to act via endothelium-dependent hyperpolarization, to activate K^+ channels, to modulate neurotransmission, and to interfere with Ca^{2+} mobilization (see Randall & Kendall, 1998). In the emerging literature, both the endothelial dependence and the involvement of cannabinoid receptors in the vasorelaxant responses are controversial.

The endogenous production of cannabinoids in the vasculature has also proved controversial, with several proposed sites. It was originally proposed that an endocannabinoid might be an endothelium-derived autacoid. In support of this, endothelium-derived hyperpolari zing factor (EDHF)-mediated relaxations are sensitive to inhibition of CB_1 receptor antagonists, although this is widely disputed. Furthermore, endothelial cells synthesise the endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG) (see Randall & Kendall, 1998). In the case of the latter it has recently been reported that stimulation of rat aortic endothelial cells with a releaser of EDHF is associated with the emergence of 2-AG (Mechoulam *et al.*, 1998). In contrast to these findings, it has been found in various vascular tissues that, although anandamide may cause hyperpolarization this is due to an endothelium-dependent action. There is clearly no consensus in the emerging literature that an endocannabinoid is an EDHF, although there is perhaps some evidence which points to them being a class of endothelium-derived vasorelaxants. It has also been proposed that endocannabinoids may be derived from white blood cells and platelets, and may play a role in the hypotension associated with haemorrhagic and endotoxic shock (Wagner *et al.*, 1997; Varga *et al.*, 1998). More recently, Ishioka & Bukoski (1999) have provided evidence that an endocannabinoid may be neuronally derived, causing vasorelaxation via K^+-channel activation.

The endocannabinoids represent a new class of vasoactive substances; their physiological actions and significance remain to be fully elucidated.

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