Poster Session 3 – Pharmacology

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Effect of diabetes on phosphatase expression levels in streptozotocin diabetic rat tissue

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Diabetes is a debilitating condition that poses a huge financial burden on the NHS. The vascular complications associated with diabetes and the identification of their molecular and biochemical mechanisms is part of the national research strategy published by the Department of Health in 2002 (Scottish Executive 2002). Phosphatases are key regulators of cell signalling cascades. A detailed knowledge of the changes that occur in response to high glucose concentration will improve our understanding of the pathogenic mechanisms involved in the development of diabetic vascular damage. Protein tyrosine phosphatase (PTP1B) has been identified as a potential therapeutic target for the treatment of diabetes due to its ability to negatively regulate insulin induced signalling (Hundahl Mőller et al 2000). Our preliminary data demonstrated reduced endogenous phosphatase levels in six-month-old diabetic rat tissue (aorta and sciatic nerve) (Weidmann et al 2001). In this study we have examined the underlying mechanisms involved in the observed changes in endogenous phosphatase in greater detail. Six male Sprague-Dawley rats received an intraperitoneal injection of streptozotocin (45 mg kg^{-1} in normal saline) compared with normal saline alone (control rats). Aortic and sciatic nerve tissue was dissected two months after diabetes induction and tissue stored at −80°C. Protein tyrosine- (PTP), serine and threonine (PP-1) phosphatase activity was measured in triplicate (Molecular Probe's RediPlate 96 EnzChek Tyrosine- and Serine/Threonine Assay Kit (R-22067; R-33700). Fluorescence intensity was measured at a wavelength of 400/30 nm/485/20 nm using an FL 600 Plate reader. The mean values for the respective test groups were compared using the Student's *t*-test after demonstration that the samples were normally distributed using Kolmogorov-Smirnov analysis. Results show that both diabetic PP-1 and PTP levels of aortic tissue are significantly less compared with healthy tissue (Table 1). The reduction in phosphatase activity indicates that changes in cell signalling processes occur at a very early stage in the disease process. The extent and nature of the observed changes depend on the type of tissue examined. Further detailed work is required to determine if the changes in cell signalling are key contributing factors in the development of diabetic long-term complications.

Table 1 Mean (s.e.m.) values between the different test groups

	Aorta (fluorescence/μg protein)		Sciatic nerve (fluorescence/μg protein)	
	Healthy	Diabetic	Healthy	Diabetic
PP-1	64.000 (±5.493)	40.000 (±6.103)	117.20 (±13.72)	99.20 (±11.88)
PTP	120.83 (±7.82)	64.33 (±5.49)	107.50 (±14.05)	81.3 (±5.9)

Hundahl Mőller, N. P., et al (2000) *Curr. Opin. Drug Discov. Dev.* **3**: 527–540
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The measurement of endotoxin on cotton fibres and implications for industrial health

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Cotton fibres become contaminated with a range of bacteria as the crop plants grow in the field. These microbes come from soil, water and other plants. Early stages of cotton processing do not include chemical washing hence the contamination persists. Many of these microbes are Gram-negative bacteria, which release endotoxin from their outer membranes. Inhalation of endotoxin causes a decrease in forced expiratory volume in one second (FEV_1), the primary targets in the lungs being the alveolar macrophages, which release an extensive inflammatory mediator cascade (Rylander 1992). The contamination entrained in the cotton fibres enters the cotton mill atmosphere along with the dust clouds created during cotton processing. Prolonged exposure to these dusty environments can result in the chronic and irreversible lung disease, byssinosis. Despite the known effects of endotoxin, there are currently no threshold limits in place for its control within the working environment. This study involved the quantification of endotoxin in numerous cotton samples from diverse countries and from within the same the country. The aim was to investigate differences in levels and hence assess risk to respiratory health and how this may vary with geographical source. Ten cotton lint samples were provided by the Liverpool Cotton Research Corporation from countries within Africa, the Middle East and North and South America. The quantification method was based on the *Limulus* Amebocyte Lysate (LAL) assay, where lysate prepared from purified blood cells (amoebocytes) from the American Horseshoe Crab (*Limulus polyphemus*) forms a gel on contact with endotoxin and glucan (see Lane et al 2004). Endotoxin was extracted from the cotton fibres into aqueous solution by 60-min vortex mixing in pyrogen-free water. Quantification was performed by monitoring the LAL reaction with aqueous endotoxin, on a plate reader via a spectrophotometer, with the addition of a glucan-blocking reagent to render the assay endotoxin-specific. The contamination level was inversely related to the time taken to reach the onset density of 0.03 optical density units, measured at 340 nm and 37°C. Unknown sample time values were read from an endotoxin standard curve, created simultaneously with known concentrations of control standard endotoxin. All cotton samples tested contained significant amounts of biological contamination. The range of endotoxin levels measured in samples from diverse countries varied from 16.0 ± 2.0 to 137.9 ± 21.6 ng g^{-1}, with cotton from Paraguay exhibiting the highest levels and the sample from Tajikistan the lowest. The mean endotoxin level in cotton samples from African countries was higher than in those from Asian countries (Table 1). This is of industrial health importance, since less developed countries are less likely to have sufficient economic means to monitor dust levels within cotton production facilities. Samples from within the same country (Sudan) had less variation in endotoxin levels ranging from 25.2 ± 1.7 to 99.3 ± 13.0 ng g^{-1}, indicating that diverse conditions during growth and harvest may affect the contamination level of cotton fibres. The application of a reproducible endotoxin quantification tool will be of use when evaluating health risks associated with specific regions.

We gratefully acknowledge the support of the Liverpool Cotton Research Corporation and the British Cotton Growing Association.

Table 1 Mean endotoxin level in cotton lint samples from different continents

Region of origin	Endotoxin (ng g^{-1})
Africa	78.2 ± 14.0
Asia	34.4 ± 12.6

Lane, S. R., Nicholls, P. J., Sewell, R. D. E. (2004) *Inhal. Toxicol.* **16**: 217–229
Rylander, R. (1992) *Tubercle Lung Dis.* **73**: 21–26

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The applicability of a glucan-specific enzyme assay for the measurement of (1-3)-β-D-glucan on cotton fibres and implications for occupational health

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(1-3)-β-D-Glucan has recently emerged as a significant agent in inhalation toxicology. Cotton fibres contain high levels of fungal contamination when harvested (Salvaggio et al 1986). Glucans are glucose polymers consisting of glucopyranosyl subunits, which occur in the cell walls of common fungi. (1-3)-β-D-Glucans have known immune stimulatory effects and upon inhalation they activate macrophages leading to the production and release of inflammatory mediators (Williams 1997). During the processing of cotton

fibres these fungi are subject to various physical stresses resulting in structural damage to hyphae and glucan release into the mill atmosphere along with cotton dust. Prolonged exposure to this agent may be a causal factor in the development of the lung disease byssinosis. The measurement of glucan levels in occupational environments is essential to evaluate potential risk of respiratory illness to cotton workers. This study analysed the application of a glucan-specific enzyme assay for use on cotton fibres and compared results for cotton samples from diverse sources. Ten cotton lint samples were provided by the Liverpool Cotton Research Corporation from countries within Africa, the Middle East and North and South America. The glucan assay was based on the *Limulus* Amoebocyte Lysate (LAL) assay, which relies on the ability of lysate prepared from purified blood cells (amoebocytes) from the American Horseshoe Crab (*Limulus polyphemus*) to coagulate on contact with glucan or endotoxin, resulting in a gel. The glucan-specific test used had been engineered by removal of the endotoxin reaction pathway by purification. Glucan-specific tests such as this are utilised clinically for the diagnosis of invasive deep mycosis. To apply the test to cotton lint, glucan was first extracted from the fibres into aqueous solution by 60-mins vortex mixing in pyrogen free water, the supernatant was then removed for analysis. Quantification was carried out by monitoring the assay on a plate reader via a spectrophotometer, where the contamination level was inversely related to the time taken to reach the onset density of 0.03 optical density units, measured at 405 nm and 37°C. Unknown time values were read from a glucan standard curve, created simultaneously with known concentrations of control standard glucan. The assay gave valid results according to the manufacturers criteria with respect to the standard curve and positive and negative controls. Aqueous extractions required 1/1000 dilution to correspond to the concentration range of the standard curve (12.5–100 pg mL^{-1}). Although there was some deviation in repeated tests from individual samples, this assay method was adequately reproducible to indicate levels of glucan contamination in cotton fibres (Table 1). All cotton samples tested were found to contain high concentrations of glucan, ranging from 294.5 ± 77.0 ng g^{-1} to 977.1 ± 249.2 ng g^{-1} fibres, with cotton from Benin having the highest and fibres from Paraguay the lowest level. This technique could be extended to assess potential risk to respiratory health in different regions, enabling exposure to be monitored.

We gratefully acknowledge the support of the Liverpool Cotton Research Corporation and the British Cotton Growing Association.

Table 1 Range of glucan levels in cotton lint samples from three representative diverse geographic regions

Country of origin	Glucan (ng g^{-1})
Benin	977.1 ± 249.2
Paraguay	294.5 ± 77.0
Syria	457.7 ± 19.9

Salvaggio, J. E., O'Neil, C. E., Butcher, B. T (1986) *Environ. Health Perspect.* **66**: 17–23
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Investigation of endogenous and H_2O_2-induced DNA damage in lymphocytes derived from schizophrenic patients and control subjects using the comet assay

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Schizophrenia, a mental disorder affecting approximately 1% of the population world-wide, typically occurs in the second or third decade of life, with individuals being affected throughout most of their life. Although the aetiology of the disorder remains unknown, investigation of the effects of oxidative stress in schizophrenia have largely focused on the determination of lipid oxidation products (Scottish Schizophrenia Research Group 2000). Oxidative damage to lipids and proteins can result in a number of pathophysiological processes while changes in DNA may alter gene expression or cause cell death and lead to genetic modification and mutagenesis. There is increasing evidence that oxidation is involved in the development of cancer and a recent study has reported an increased overall risk of cancer in patients with schizophrenia compared WITH that of the general population (Lichtermann et al 2001). To date, only one study has examined the effects of oxidative insult on the cellular DNA of a Greek sample of male schizophrenic patients and controls, although no significant difference was reported (Psimadas et al 2004). However, the study reported here is the first to investigate the effects of oxidative stress in a British sample of male and female schizophrenic patients and controls on the level of DNA damage. In this study a comparison of endogenous and hydrogen peroxide (H_2O_2)-induced DNA damage in schizophrenic and normal lymphocytes was undertaken. Ethical approval was obtained from the LREC and all procedures carried out in accordance with the Helsinki Declaration (1975) and the Data Protection Act (1998). Schizophrenic patients ($n = 15$) and apparently healthy controls ($n = 17$) were recruited and informed consent obtained from ward patients and staff at the New Craigs Hospital, Inverness. The trial protocol was reviewed by ward consultants at the hospital and participants matched for age, gender and smoking status. Venous blood (9 mL) was collected from 32 subjects (27 smokers, 5 non-smokers), comprising 11 male and 4 female schizophrenic patients, (average age $= 37.9 \pm 11.0$) and 12 male and 5 female healthy controls (average age $= 38.9 \pm 9.2$). Lymphocytes were separated by centrifugation and either treated with 0, 50 and 200 μM H_2O_2 or cryopreserved at -80°C. The single cell gel electrophoresis assay (comet assay) was used to evaluate DNA damage; cells were embedded in agarose on a microscope slide, lysed and immersed in alkaline buffer to enable DNA unwinding. Nucleoids were electrophoresed, washed and stained and scored visually using a fluorescence microscope. One-hundred random comets from each gel were scored by an examiner who was blinded to treatment group and were classified into one of five classes according to the relative intensity of fluorescence in the tail with a value of 0–4 (0 = undamaged, 4 = maximally damaged). Preliminary data suggests no significant decrease in the level of endogenous DNA damage between schizophrenic patients and the control group. The susceptibility of lymphocytic DNA to an oxidative challenge (H_2O_2) is currently under investigation. Further understanding of the role that oxidative stress may play will be valuable for developing new and innovative therapeutic strategies for schizophrenia and associated co-morbidities.

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Psimadas, D., et al (2004) *Cancer Lett.* **204**: 33–40
Scottish Schizophrenic Research Group (2000) Br. J. Psychiatry **176**: 290–293

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Synthesis and effects of four novel dihydropyridines on rat atrium and colon smooth muscle

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The influx currents of calcium through L-type voltage dependent calcium channels play a crucial role in modulation of smooth muscle contractions. Therefore, the dihydropyridine compounds, as L-type calcium channel blockers, have received much attention in therapeutics. Numerous investigations are carried out to design novel dihydropyrines with more selectivity and less adverse effects. One of the adverse effects associated with nifedipine, which is a classical dihydropyridine, is its negative inotropic effects on the heart. In this study a series of novel 4-(3-benzyl-2-alkylsulfanyl-3H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dialkyl esters (**4a–d**) were synthesized in which o-nitropheny of nifedipine has been replaced with benzyl imidazolyl substituent. Initially 1-benzyl-2-mercapto-imidazole-5-methanol (**1**) was synthesized from benzylamine hydrochloride and dihydroxyacetone then it was alkylated to 2-alkylthio-1-benzylimidazole-5-methanol (**2**). Oxidation of 2 gave carbaldehyde (**3**). Compound **3** was converted to the novel 4-(3-benzyl-2-alkylsulfanyl-3H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dialkyl esters (**4a–d**) through classical Hantzsch method. The compounds were characterized by ^{1}H NMR and IR spectroscopy (Hadizadeh et al 2002). All test compounds (**4a–d**), which differed only at alkyl groups, showed positive inotropic effects on the isolated rat left atrium (Vogel & Vogel 1997). This was in contrast

to classical dihydropridines of which nifedipine is the prototype. EC50 values were defined as concentration needed to increase percent of contraction by 50% and IC50 values were defined as concentrations needed to decrease percent of contraction by 50%. 4-(3-Benzyl-2-ethylthio-3H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydro pyridine-3,5-dicarboxylic acid diethyl ester (**4a**) was the most potent and its EC50 was found to be 4×10^{-5} M (positive inotropic effect). In contrast, nifedipine decreased percent of contraction and its IC50 was found to be 5×10^{-8} M (negative inotropic effect). Test compounds (**4a**–**d**) decreased contractile responses of colon muscle to KCl (Vogel & Vogel 1997). Compound **4a** decreased percent of contraction of colon muscle in presence of KCl and its IC50 was 6×10^{-5} M. IC50 for nifedipine was 5×10^{-8} M. Since these compounds are analogues of nifedipine, their effects are most likely due to modulation of L-type calcium channels. It may be concluded that replacement of o-nitophenyl substituent in nifedipine with imidazolyl substituent may cause some partial calcium channel agonist properties at the heart muscle while calcium channel antagonist properties at other smooth muscles (colon muscle) persists. These compounds may be effective in patients with congestive heart failure.

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Effects of phytoestrogens on the contractile activity of rat blood vessels

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Genistein (GEN) a tyrosine kinase inhibitor and its analogue daidzein (DAID), devoid of this activity, are plant-derived isoflavonoids that possess weak, oestrogen-like relaxant effects on blood vessels. Populations consuming diets rich in these phytoestrogens appear to have reduced cardiovascular disease risks and it has been proposed that GEN could substitute for oestrogens in hormone replacement therapy. The mechanism by which phytoestrogens relax blood vessels remains controversial. Lee & Man (2003) observed genistein-induced relaxation, which was independent of the endothelium, whereas Mishra et al (2000) reported endothelium-dependent relaxation by both genistein and daidzein. This work compares relaxant actions of GEN and DAID with those of the endogenous oestrogen, 17β oestradiol (EST), in two different blood vessels of the rat. Aortic rings, intact or without endothelium, from male Hooded-Lister rats (250–350 g) were studied in Krebs' solution (37°C, 95% O_2, 5% CO_2) containing indomethacin (10 μM) (KS) under 2 g tension. Functional aortic endothelium was confirmed by relaxation (>30%) to acetylcholine (1 μM) following contraction by KCl (60 mM). Portal veins (PV) were placed in KS under 0.5 g tension. Concentration–response curves to KCl (10–100 mM) were constructed in each tissue in the presence or absence of EST (10–20 μM), GEN (20–40 μM) or DAID (20–40 μM), N = 4–6. Genistein caused concentration-related reduction in responses to KCl in intact aorta and PV, maximum relaxation being $32 \pm 3.6\%$ ($P < 0.01$) and $51 \pm 6.0\%$ ($P < 0.001$) respectively. DAID-induced relaxation was weak in intact aorta ($21 \pm 2.5\%$, $P < 0.05$) but marked in PV, maximal relaxation was $62 \pm 4.0\%$ ($P < 0.001$). GEN and DAID were ineffective in de-endothelialised aorta. Higher phytoestrogen concentrations were not used due to vehicle effects. EST caused greater relaxation than phytoestrogens in all tissues (Table 1). The results show that actions of GEN and DAID differ from those of EST, causing weak relaxation in intact aorta but no relaxation following endothelium removal. Both agents significantly relaxed PV; DAID appeared to be more effective, suggesting that tyrosine kinase inhibition plays no part in the relaxation mechanism. EST was considerably more effective than the phytoestrogens, producing comparable relaxation in all tissues. We conclude that phytoestrogen-induced relaxation is weak and endothelium-dependent in rat aorta, but not in PV. GEN and DAID-induced relaxation in PV is substantial and, like EST, relaxation is independent of the endothelium as in PV, the longitudinal muscle studied in experiments is separated from endothelium by circular muscle. These results show that relaxation elicited by phytoestrogens does not depend on the presence of endothelium in all types of blood vessel.

Table 1 Comparison of maximal relaxation by 17β oestadiol (EST), genistein (GEN) and daidzein (DAID) of KCl-induced contraction in aorta and portal vein

Agent (μM)	Intact rings	Portal vein	De-endothelialised rings
GEN 20	10 ± 2.5*	14 ± 7.5*	NS
GEN 40	32 ± 3.6**	51 ± 6.0***	NS
DAID 20	NS	45 ± 2.5**	NS
DAID 40	21 ± 2.5*	62 ± 4.0***	NS
EST 10	48 ± 3.5**	61 ± 3.7***	30 ± 6.0**
EST 20	71 ± 3.4***	86 ± 9.0***	77 ± 7.5***

Figures represent % reduction in Emax. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. NS, no significant effect.

Lee, M. Y. K., Man, R. Y. K. (2003) *Eur. J. Pharmacol*. **481**: 227–232
Mishra, S. K., et al (2000) *Cardiovasc. Res*. **46**: 539–546

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Comparison of the relaxant properties of 17β oestradiol and oestrogen metabolites on smooth muscle

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Endogenous oestradiol exerts complex actions on blood vessels which may contribute to protection of pre-menopausal women from coronary heart disease (Barrett-Connor 1997). However, in the body 17β oestradiol (EST) is converted to both oestrogenic and non-oestrogenic metabolites which potentially could also affect blood vessels. It has been shown that 2 hydroxyestradiol and 2 methoxyestradiol are more potent than oestradiol in inhibiting vascular muscle growth; 2 methoxyestradiol also has marked antimitogenic, antitumour and antiangiogenic activity (Dubey et al 2002). Thus the cardiovascular protective effects of oestradiol may be due, in part, to the actions of metabolites. In this work we studied the actions of 2-methoxyestradiol (2ME), comparing its relaxant effect with that of EST, together with the metabolite and major postmenopausal oestrogen, oestrone (OST), on two smooth muscle preparations, vascular and intestinal. Portal veins (PV) or segments of terminal ileum from male Hooded-Lister rats (250–350 g) were set up in Krebs' solution (37°C, 95% O_2, 5% CO_2) containing 10 μM indomethacin, under 0.5 g or 1 g tension, respectively. In PV a control concentration response curve to KCl was constructed and repeated following incubation with EST (4–8 $\mu\mu$), 2ME (4–8 μM) or OST (4–8 μM) for 20 min. Control concentration–response curves were constructed to carbachol in the ileum and repeated following incubation with EST (4–8 $\mu\mu$), 2ME (4–8 $\mu\mu$) or OST (4–16 $\mu\mu$) for 20 min. (N = 6). In PV, EST (4–8 $\mu\mu$) shifted the KCl concentration–response curve rightwards, causing a concentration-related reduction in Emax, maximum relaxation being $61.3 \pm 1.4\%$ ($P < 0.001$). 2ME and OST (4–8 $\mu\mu$) caused small rightward shifts in the carbachol concentration–response curve and a maximum reduction in Emax of $48.9 \pm 4.4\%$ and $37.6 \pm 4.3\%$ ($P < 0.001$), respectively. In ileum EST, 2ME (4–8 μM) and OST (8 μM) caused relaxation which was similar but, particularly in the case of oestrone, smaller than that observed in the portal vein: maximum reduction in Emax was $53.9 \pm 6.6\%$, $28.0 \pm 5.3\%$ and $15.8 \pm 3.4\%$ ($P < 0.01$), respectively. In further experiments potential effects of 2ME on calcium mobilisation in PV, a tissue that is exclusively dependent on extracellular Ca^{++} for contractile activity, were studied. Cumulative concentration–response curves to calcium were constructed in Ca^{++}-free depolarising solution containing 32 mM KCl and indomethacin (10 μM) in the absence or presence of EST or 2ME (4–8 $\mu\mu$). Both agents displaced concentration–response curves rightwards, considerably reducing Emax. Maximum reduction in Emax was similar, being $41.5 \pm 4.5\%$ and $46.0 \pm 4.6\%$ for EST and 2ME (8 μM) ($P < 0.01$), respectively. These results show that endogenous oestradiol is more effective in relaxing both types of smooth muscle than either metabolite. The relaxant effects of 2ME were not significantly different from those of OST in portal vein, but were greater than OST in intestinal muscle. As previously reported for oestradiol and diethylstilboestrol, 2ME appears to relax portal vein by reducing Ca^{++} influx. We conclude that oestrogen metabolites, like oestradiol, can relax different types of smooth muscle and contribute to vasodilator actions of both endogenous oestrogens and oestrogens in hormone replacement therapy. Although 2ME is more potent than oestradiol in inhibiting vascular muscle growth and proliferation, oestradiol is the more effective relaxant of vascular muscle.

Barrett-Connor, E. (1997) *Circulation* **95**: 252–264
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The role of extracellular calcium in oestrogen-induced relaxation of isolated rat aorta

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Sadly Dr Evans died soon after this abstract was written.

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Experimental and epidemiological studies indicate that oestrogens exert complex actions on intact blood vessels, including dilator actions, which may contribute to their reported cardiovascular protective actions (Grodstein et al 2000). Many mechanisms have been proposed to account for oestrogen-induced dilatation, including changes in calcium influx, enzymes and ion channel activity and nitric oxide (NO) release from the endothelium. However, the mechanism remains controversial. We showed previously that NO release contributes significantly to oestrogen-induced relaxation in rat aorta but apparently not in portal vein (McCurrie et al 2003). In these experiments we investigated possible effects of oestrogen on calcium influx by comparing the ability of 17β oestradiol (EST) to relax aorta in the absence of extracellular calcium with that of the L type calcium channel blocker, nifedipine (NIF). Intact aortic rings prepared from male Hooded-Lister rats (250–400 g) were placed in Krebs' solution containing indomethacin (10 μM) under 2 g tension (37°C, 95% O_2, 5% CO_2). Cumulative concentration–response curves to phenylephrine (PHE, 0.01–10 μM) were constructed in the absence or presence of EST (2–10 μM). Following incubation of rings for 120 min in calcium (Ca^{++})-free Krebs', KCl (80 mM) was added to depolarise the preparation. After 15 min, cumulative Ca^{++} concentration–response curves were constructed in the absence or presence of EST (10–20 μM) or the calcium channel blocker, NIF (0.01–0.1 μM). In further experiments, following incubation of aorta in Ca^{++}-free Krebs for 120 min with addition of EDTA (1 mM) to remove any remaining extracellular Ca^{++}, cumulative concentration–response curves to PHE were constructed in the absence or presence of EST (20 μM) or NIF (0.1 μM). Relaxation was expressed as % reduction of contraction, N = 4–6. EST (2–10 μM) caused small rightward shifts of the PHE concentration–response curve and concentration-related relaxation in normal Krebs' solution, maximum 48 ± 7% ($P < 0.001$). In Ca^{++}-free solution a higher concentration of EST was required to obtain significant relaxation. EST (20 μM) reduced the Ca^{++}-induced Emax by 15 ± 3% ($P < 0.01$), with displacement of the concentration–response curve. NIF (0.01–0.1 μM) caused large rightward displacements in the Ca^{++} concentration–response curve, the highest concentration used reduced Emax by 30 ± 4% ($P < 0.001$). In the absence of extracellular Ca^{++}, EST (20 μM) greatly reduced contractions induced by PHE, decreasing Emax by 55 ± 6% ($P < 0.001$), whereas NIF was without effect on PHE-induced contraction in this solution. These results show that EST relaxes both PHE and Ca^{++}-induced contraction in aorta, the latter being a relatively modest effect. EST relaxed PHE-induced contraction in both normal and Ca^{++}-free Krebs. On the other hand, the L-type Ca^{++} channel blocker, nifedipine, a potent relaxant of contraction elicited by a wide variety of agonists including KCl, PGF2α and PHE in rat aorta, was completely ineffective in relaxing contraction when extracellular Ca^{++} was removed. Since EST-induced relaxation was retained in the absence of extracellular Ca^{++}, a situation in which the possibility of a drug exhibiting calcium channel blocking activity is eliminated, we conclude that changes in Ca^{++} influx play only a small part in the relaxant effects of oestradiol in the rat aorta.

Grodstein, F., et al. (2000) *Ann. Intern. Med.* **133**: 933–941
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(+)-Catechin mediates its spasmolytic effect via blockade of calcium influx

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Flavonoids are polyphenolic substances found abundantly throughout the plant kingdom. Many herbal remedies used in different cardiovascular, gastrointestinal and respiratory diseases contain flavonoids. One such flavonoid is (+)-catechin, a catechol derivative found in plants like *Areca catechu, Acacia catechu, Piper betle, Uncaria gambir*, etc. These, along with some other plants containing (+)-catechin, are used traditionally in hyperactive states of the gastrointestinal and respiratory systems such as in diarrhoea and asthma (Gathercoal & Wirth 1947). Here, in this study, we report the spasmolytic activity of (+)-catechin in different gastrointestinal and respiratory smooth muscle preparations possibly mediated via a calcium channel blocking (CCB) mechanism. Segments of rabbit jejunum, rat stomach fundus, guinea-pig ileum and trachea were isolated and suspended separately in 10-mL tissue baths containing Tyrode's or Kreb's solution. Solutions were aerated with carbogen gas (5% CO_2 in oxygen) while the temperature was fixed at 37°C. Responses were recorded on Harvard Student Oscillographs for gastrointestinal tissues while on Grass Polygraph for the tracheal tissues. Catechin dose-dependently (1–30 mM) relaxed the spontaneously contracting isolated rabbit jejunum. When tested against high K^+ (80 mM)-induced contractions, catechin caused a dose-dependent (0.3–3 mM) relaxation suggestive of CCB activity. This CCB activity was confirmed when pre-treatment of the tissue with catechin (1–3 mM) shifted the Ca^{++} dose–response curves to the right, similar to that produced by verapamil, a standard calcium channel blocker. When tested on other gut preparations, like fundus or ileum, it did not show any activity on the resting base line but when tested on high K^+ (80 mM)-induced contraction, caused a dose-dependent (0.3–3 mM) relaxation. In the tracheal strips, the compound exhibited non-specific bronchodilation (1–10 mM) when tested against carbachol (1 μM) and high K^+-induced contractions, a characteristic of calcium antagonists. No stimulant effect of the compound was seen on the resting base line of the tracheal preparation. These results show that catechin exhibits non-specific spasmolytic activity in the different gastrointestinal and airway smooth muscles, possibly mediated via calcium channel blocking mechanism. Thus, the presence of catechin, in plants used in hyperactive states of the gut or airways, may be the contributing factor for the therapeutic success of these plants.

Gathercoal, E. N., Wirth, E. H. (1947) In: *Pharmacognosy*. Lea & Febiger, Philadelphia, p. 608

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The anti-emetic action of cannabinoid extracts containing high levels of cannabidiol and cannabidiolic acid on motion induced emesis in *Suncus murinus*

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A cannabinoid extract containing >6.3% cannabidiol (CBD) and <2% tetrahydrocannabinol (THC) was found to significantly attenuate motion-induced emesis in *Suncus murinus* (Javid et al 2002). It was further identified that the CBD content of the extract was composed of 7 parts cannabidiolic acid (CBDA) and 1 part CBD. The aim of this work was to further investigate the antiemetic potential of CBD, CBDA and a botanical drug substance (BDS) containing high levels (63.8%) of CBD (BDS (CBD)). Adult *Suncus murinus*, 61.4 ± 1.6 g, were administered intraperitoneally with either CBD (0.5, 1 or 2 mg kg^{-1}), CBDA (0.02, 0.1 or 0.5 mg kg^{-1}), BDS (CBD) (0.25, 0.5 or 1 mg kg^{-1}) or vehicle. The animals were placed individually in transparent cages (100(W) × 150(L) × 150 (H) mm) of a motion stimulator and observed for 45 min for any behavioural changes before motion was applied, at a frequency of 1 Hz and amplitude of 40 mm for 10 min. The number of emetic episodes and latency of onset to the first episode (s) were recorded. Data were expressed as the mean ± s.e.m. of n = 5 or 6 and analysed using Student's *t*-test with $P < 0.05$ being taken as a significant difference between treatments. The number of emetic episodes was reduced by 67% (from 11.5 ± 2.3 to 3.8 ± 1.1 emetic episodes ($P < 0.05$)) and 94% (from 12.0 ± 2.2 to 0.8 ± 0.8 emetic episodes ($P < 0.001$)) following the intraperitoneal injection of 0.1 and 0.5 mg kg^{-1} CBDA, respectively, as compared with the vehicle-treated animals. Administration of 0.1 and 0.5 mg kg^{-1} CBDA significantly ($P < 0.01$) increased the latency of onset to the first emetic episode from 115.3 ± 23.2 to 400.2 ± 80.3 s and 153 ± 31.9 to 500.4 ± 83.0 s, respectively, as compared with the vehicle-treated animals. Animals injected with 0.02 mg kg^{-1} CBDA showed a comparable emetic response to motion to the vehicle-treated animals ($P > 0.05$). In shrews challenged with 0.5, 1 and 2 mg kg^{-1} CBD the number of emetic episodes and latency of onset to the first emetic episode were comparable with those in the vehicle-treated animals ($P > 0.05$). Following the administration of 0.25, 0.5 and 1 mg kg^{-1} BDS (CBD) both the number of emetic episodes and latency of onset of emesis were comparable with values observed in the vehicle-treated animals ($P > 0.05$). In summary, the administration of CBDA dose-dependently attenuated the emetic response to motion in *S. murinus*; CBD and BDS (CBD) were ineffective. The data indicates an antiemetic pharmacological activity of a cannabinoid carboxylic acid in *Suncus murinus*.

Javid, F. A., et al (2002) *FENS Abstr* A142.14.

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The effect of cannabinoid extracts containing high levels of tetrahydrocannabinol and tetrahydrocannabinolic acid on motion induced emesis in *Suncus murinus*

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It is well established that tetrahydrocannabinol (THC) has anti-emetic actions against nausea and vomiting induced by cytotoxic-chemotherapy (Ward & Holmes 1985) and radiation therapy (Priestman et al 1987). However, its effect on motion-induced emesis is unknown. Adult *Suncus murinus* (59.7 ± 1.4 g) were administered intraperitoneally with either THC (0.25, 0.5 or 1 mg kg^{-1}), tetrahydrocannabinolic acid (THCA) (0.25, 0.5 or 1 mg kg^{-1}), a botanical drug substance containing 73% THC (BDS (THC)) (0.25, 0.5 or 1 mg kg^{-1}) or vehicle. The animals were placed individually in transparent cages ($100(W) \times 150(L) \times 150$ (H) mm) of a motion stimulator and observed for 45 min for any behavioural changes before motion was applied at a frequency of 1 Hz and an amplitude of 40 mm for 10 min. The number of emetic episodes and latency of onset to the first emetic episode (s) were recorded. Data were expressed as the mean ± s.e.m. of n = 5–10 and analysed using Student's *t*-test with $P < 0.05$ being taken as a significant difference between treatments. The number of motion-induced emetic episodes and latency of onset of emesis recorded following pre-treatment with 0.25, 0.5 and 1 mg kg^{-1} THC were comparable with the vehicle-treated animals ($P > 0.05$). In shrews challenged with 0.25, 0.5 and 1 mg kg^{-1} THCA the number of emetic episodes in response to motion were comparable with values observed in the vehicle-treated animals ($P > 0.05$). However, the latency of onset to the first emetic episode was reduced from 395.5 ± 80.5 to 191.8 ± 32.1 s ($P < 0.05$) and from 393 ± 63.9 to 125.2 ± 29.9 s ($P < 0.01$) following the intraperitoneal administration of 0.25 and 0.5 mg kg^{-1} THCA, respectively, as compared with the vehicle-treated animals. Animals injected with 1 mg kg^{-1} THCA showed a comparable latency of onset to the first emetic episode as the vehicle-treated animals ($P > 0.5$). Intraperitoneal injection of 0.25, 0.5 and 1 mg kg^{-1} BDS (THC) did not affect motion-induced emesis in *S. murinus* compared with the vehicle-treated animals ($P > 0.05$). In contrast to its antiemetic effect on drug-induced emesis, it appears that THC may not be anti-emetic to motion-induced emesis in *S. murinus* using the described parameters.

Priestman, et al (1987) *Clin. Radiol*. **38**: 543–544
Ward & Holmes (1985) *Drugs* **30**: 127–144

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ACE-inhibitory activity of captopril methyl ester prodrug

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Previously Moss et al (2003) have described the synthesis and characterisation of a range of carboxyl-ester captopril prodrugs. Delivery of the angiotensin converting enzyme (ACE) inhibitor, captopril, would benefit from the zero-order kinetics associated with the transdermal route. The transdermal/prodrug route may also avoid the first dose hypotensive effect of ACE inhibitors therapy (Weber et al 2002). These prodrugs have been designed rationally via a QSAR approach to have range of log P values from 0.84 to 3.66 to have significantly enhanced percutaneous absorption. The metabolic fate of carboxyl-ester captopril prodrugs in either in-vivo or in-vitro is unknown and is under investigation by this group. As part of this investigation we have examined the ACE-inhibitory effect of the methyl ester prodrug (MEP) to ensure activity is not adversely affected by the liberation of the parent drug, captopril. It has been reported that the carboxylic acid functionality in captopril is essential for good binding (Attwood et al 1984). Synthetic studies have shown that the stereochemical configuration of the captopril moiety has been maintained (Moss et al 2003). The first candidate that has been chosen for this is the methyl ester prodrug, being the simplest of all the prodrugs. Captopril and MEP were examined quantitatively for their ability to inhibit angiotensin I-induced contractions of rat aortae in-vitro. Thoracic aortae obtained from male Wistar rats (170–230 g) were cut into helical spirals of 50–70 mm in length. Each spiral was mounted under 500 mg passive tension in Krebs ringer solution maintained at 37°C and gassed with 95% O_2 5% CO_2. Contractile responses to angiotensin I and II were recorded isometerically. Angiotensin II produced dose-related contraction which were unaffected by prior administration of captopril. Angiotensin-I induced contractions, however, were diminished in the presence of captopril, indicating the necessity of the conversion of angiotensin I to angiotensin II by endothelial ACE, and totally inhibited at 10^{-8} M captopril. MEP similarly reduced the responses to angiotensin I with total inhibition at 10^{-7} M. This suggests that the MEP retain some of the ACE-inhibitory properties of captopril despite the masking of the carbonyl functionality and upon its metabolism to captopril it will return to the activity associated with that. The clinical and pharmaceutical applications of MEP and the other prodrugs are ongoing in our laboratories.

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Moss, G. P., et al (2003) *J. Pharm. Pharmcol*. **55S**: 20–21
Weber, S., et al (2002) *Am. Heart J*. **143**: 313–318