

Poster Session 3 – Pharmacology

226
Role of nicotinic receptors in hypoxia-induced cell death in primary cortical cultures

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One of the potential risk factors for neurological dementias like Alzheimers's disease (AD) is the transient incidence of hypoxia/ischaemia (HI) as a result of strokes or head injuries (Nicholl et al 1995). This suggests that HI may trigger amyloidogenesis via a signal transduction cascade that mediates neurodegeneration. Progression of AD is also characterised by a marked cholinergic dennervation due loss of basal cholinergic nuclei and neurones expressing the nicotinic acetylcholine receptors (nAChRs). This cholinergic pathway also happens to be highly susceptible to hypoxia (Neiber 1999). However, the relationship between hypoxia and nAChRs in the neurodegenerative process has never been fully explored. (−)-Nicotine, a prototypical, non-selective agonist of these nAChRs has been shown to have a neuroprotective role, in-vitro (Dajas-Bailador et al 2000) as well as in-vivo (Maggio et al 1998). This study was therefore aimed at examining the role of nicotinic receptors in mediating hypoxia-induced neuronal death.

Mature rat primary neocortical cultures (8–10 day) were subjected to 4h hypoxia (0.1% O_2) ± nicotine/nicotinic antagonists. Cytotoxicity was determined ~18–20 h following treatment using the MTT assay. Apoptosis was determined by TUNEL staining 18–20 h following treatment. The effect of nicotinic drugs on DNA damage was monitored using the alkaline SCGE (Comet assay). Activation of caspases3/7 was measured using the Apo-1 homogenous caspase 3/7 assay (Promega).

HI caused a 44% increase in cell death following 4 hr hypoxia as analysed by the MTT assay. Results from the TUNEL staining suggest that the nature of cell death is primarily apoptotic. Increased apoptosis was indirectly assessed by measuring the levels of caspase 3/7 which increased 2 fold following 4-h hypoxia. Cytotoxicity data correlated with Comet assay analysis, showing that hypoxia increased induction of DNA double strand breaks whereas the presence of nicotine (10 μM) attenuated this effect. (−)-Nicotine was neuroprotective from 0.5 to 100 μM with maximum protection being observed at 100 μM. To identify the specific nAChR subtypes involved in neuroprotection, cultures were pre-exposed to nAChR antagonists for 30 min followed by 10 μM (–)-nicotine exposure during hypoxia. 1 μM dihydro-β-erythroidine (DHβE) (non-α7nAChR selective) as well as α-bungarotoxin (selective for α7nAChR) inhibited this neuroprotective effect, suggesting the possible involvement of multiple nAChR subtypes in neuroprotection. Muscarinic AChR antagonists like atropine had no effect on hypoxia-induced cell death. The results of this study show that the nature of hypoxia-induced cell death is primarily apoptotic, and that (−)-nicotine offers protection against hypoxia-induced neurotoxicity, which may be mediated by both the α7nAChR as well as α4β2 nAChR.

Dajas-Bailador, F. A., Lima, P. A., Wonnacott, S. (2000) *Neuropharmcology* **39**: 2799–2807

Maggio, R., Riva, M., Vaglini, F., et al (1998) *J. Neurochem.* **71**: 2439–2446

Neiber, K. (1999) *Pharmacol. Ther* **82**: 71–86

Nicoll, J. A. R., Roberts, G. W., Graham, D. I. (1995) *Nature Med.* **1, 2**: 135–137

227
Anti-inflammatory properties of *Hypoxis hemerocallidea* corm extracts in rats

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The tuberous rootstock (i.e., the corm) of *Hypoxis hemerocallidea* (Fisch. & Mey.) (family: Hypoxidaceae), locally known in South Africa as African Potato (AP), is frequently referred to as the miracle or wonder plant medicine, and used for a variety of human ailments, including arthritis and other inflammatory conditions. This study was, therefore, undertaken to examine the anti-inflammatory properties of AP extracts in the rat model of inflammation. Groups of AP extract- and distilled-water-treated male, young adult, Wistar rats (250–300 g) were used. Increases in rat hind paw linear circumferences induced by subplantar injections of fresh egg albumin, a cheap phlogistic agent (Ekpendu et al1994; Muko & Ohiri 2000), have been examined in this study. Acute inflammation was induced in each of the test rats by injecting 0.5 mL kg^{-1} of fresh egg albumin on the subplantar surface of the right hind paw. Linear paw oedema was assessed for 3 h at 30-min intervals, following the administration of the phlogistic agent. The increases in right paw circumferences were compared with those of the contra-lateral, non-injected left hind paw circumferences. Oedema was assessed in terms of the difference in the zero-time linear circumference of the injected hind paw and its linear circumference at time t (i.e., 30, 60, 90, 120, 150 and 180 min) after fresh egg administration. Aqueous and methanolic extracts of AP were separately administered orally at a dose of 500 mg kg^{-1} to each of the rats in the test groups, 1 h before inducing inflammation with the injection of fresh egg albumin. Aspirin (100 mg kg^{-1} p.o.) was used as the reference anti-inflammatory agent for comparison. Rats in the control group received distilled water (2 mL kg^{-1} p.o.) only. Percentage inflammation (oedema) was calculated by the formula: Co/Ct × 100/1, while percentage inhibition of the oedema was calculated from the formula: (Co − Ct/Co) × 100/1, where Co is the average inflammation (paw circumference) of the control rats at a given time and Ct is the average inflammation of the extract- or aspirin-treated rats at the same time. Subplantar injections of fresh egg albumin (0.5 mL kg^{-1}) produced marked, sustained and progressive increases in the hind paw linear circumferences of the control (untreated) rats. Maximal swelling (oedema) was obtained approximately 90 min following fresh egg albumin administration. AP aqueous and methanolic extracts (500 mg kg^{-1} p.o.), like aspirin (100 mg kg^{-1} p.o.), produced time-related, significant reductions ($P < 0.05$–0.001) of the fresh egg albumin-induced acute inflammation of the rat hind paw. However, the anti-inflammatory effect of the extracts was found to be approximately 7–15 times less than that of aspirin. On average, however, the methanolic extract of AP produced a relatively stronger anti-inflammatory effect than the aqueous extract. The experimental evidence obtained in this study indicates that AP possesses anti-inflammatory activity, and thus lends credence to the folkloric use of the herb in the management of arthritis and other inflammatory conditions in some communities of South Africa.

Ekpendu, T. O. et al (1994) *Int. J. Pharmacogn.* **32**: 191–196

Muko, K. N., Ohiri, F. C. (2000) *Fitoterapia* **71**: 65–68

228
Hypoglycaemic effect of *Clausena anisata* (Willd) Hook methanolic root extract

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Various morphological parts of *Clausena anisata* (Willd) Hook (family: Rutaceae) have been reported to be useful as effective remedies against an array of disorders in man, including diabetes mellitus (Watt & Breyer-Brandwijk 1962; Hutchings et al1996). The core aim of this study was to examine the hypoglycaemic effect of this South African flowering plant's methanolic root extract in normal and diabetic rats, with a view to providing a pharmacological rationale for the use of *Clausena anisata* root in the management of adult-onset, type-2 diabetes mellitus in some communities of South Africa. Young adult male Wistar rats (250–300 g) were used. Diabetes mellitus was induced in the group of diabetic test rats by intraperitoneal injections of streptozotocin (STZ, 90 mg kg^{-1}). In one set of experiments, graded doses of *Clausena anisata* methanolic root extract (CAME, 100–800 mg kg^{-1} p.o.) were administered to both fasted normal and fasted diabetic rats. In another set of experiments, 800 mg kg^{-1} (p.o.) of CAME, a dose of the plant extract which

produced maximal hypoglycaemic effect in both fasted normal and fasted diabetic rats in our previous set of experiments, was used. The hypoglycaemic effect of this single dose of *Clausena anisata* root methanolic extract (CAME, 800 mg kg^{-1} p.o.) was compared with those of insulin (5 μU kg^{-1} s.c.) and glibenclamide (0.2 mg kg^{-1} p.o.) in both fasted normal and fasted diabetic rats. Following acute treatment, relatively moderate-to-high doses of CAME (100–800 mg kg^{-1} p.o.) produced dose-dependent, significant reductions ($P < 0.05$–0.01) in the blood glucose concentrations of both fasted normal and diabetic rats. On their own, both insulin (5 μU kg^{-1} s.c.) and glibenclamide (0.2 mg kg^{-1} p.o.) produced significant reductions ($P < 0.01$–0.001) in the blood glucose concentrations of fasted normal and fasted diabetic rats. At a dose of 800 mg kg^{-1} (p.o.), CAME reduced the mean basal blood glucose concentrations of fasted normal and fasted diabetic rats by 57.52% and 51.30%, respectively. Since methanol extracts of plants are usually known to contain many chemical compounds, each of which is capable of producing specific biological activity via different mechanisms, it is difficult to draw any logical conclusion on the mechanism of the hypoglycaemic effect of such a diverse mixture of chemical compounds contained in the *Clausena anisata* root methanolic extract used in this study. While it is possible that the hypoglycaemic effect of the plant extract may be due, at least in part, to its terpenoid and coumarin contents, the mechanism of its hypoglycaemic action remains largely speculative. It is, however, unlikely to be due to the stimulation of pancreatic β-cells and subsequent secretion of insulin. Although *Clausena anisata* methanolic root extract is less potent than insulin as an antidiabetic agent, the results of this experimental animal study indicate that the herb possesses hypoglycaemic activity, and thus lend credence to the suggested folkloric use of *Clausena anisata* root in the management or control of adult-onset, type-2 diabetes mellitus in some communities of South Africa.

Hutchings, A., et al (1996) *Zulu medicinal plants – an inventory*. University of Natal Press, Pietermaritzburg, South Africa, pp 153–154

Watt, J. M., Breyer-Brandwijk, M. G. (1962) *The medicinal and poisonous plants of Southern and Eastern Africa*. 2nd Edn, Livingstone, London

229
The kidney protective effect of vitamin C and hydrocortisone in allopurinol-treated mice

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It is well known that allopurinol reduces the uric acid levels in gout patients and may be used throughout life. Chronic use of this drug causes accumulation in kidney tissues and it could produce nephrotoxicity or kidney failure (Clark 1991; Hardman 1995). In this study we tried to find out the protective effect of vitamin C and hydrocortisone in toxicity induced by allopurinol.

In this investigation three positive control groups of mice received allopurinol in doses of 10 20 and 40 mg kg^{-1} for a period of 12 days. Dose-respondent effects were seen and 40 mg kg^{-1} was chosen for subsequent studies. The negative control group received normal saline. On day 12, the first group of test mice received vitamin C in dose of 4 mg kg^{-1} and after 2 h the last dose of allopurinol was administered in dose of 40 mg kg^{-1}. Similarly, after 12 days the second group of test mice received hydrocortisone in a dose of 3 mg kg^{-1} and after 2 h the last dose of allopurinol was administered in dose of 40 mg kg^{-1}. Twenty-four hours later, the mice were sacrificed and blood was withdrawn. Creatinine and BUN were measured and kidney tissue was used for histopathological examination.

The level of BUN and creatinine were 29.8 ± 0.374 and 0.9 ± 0.55, respectively, significantly improved over the control group ($P < 0.05$). Also, histopathological examinations of kidney were studied and confirmed the above data. Results in the group receiving allopurinol and vitamin C showed that interstitial nephritis vanished and little congestion remained. In the group receiving hydrocortisone and allopurinol, congestion and interstitial nephritis significantly decreased.

It is clear that the allopurinol effect is dose dependent and it causes kidney toxicity but when we use hydrocortisone and vitamin C they protected against the kidney toxicity as histopathological studies and BUN and creatinine levels showed, as compared with the positive control group.

Clark, et al (1991) *Goth medical pharmacology*. 39th edn

Hardman, J. G., et al (1995) *The pharmaceutical basis of therapeutics* 9th edn, McGraw Hill, New York

230
Effects of cis-oleamide and carbamazepine on evoked epileptiform activity induced by 4-aminopyridine in CA1 neurons of the rat hippocampal slice

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The sleep lipid cis-oleamide (cOA) is a positive modulator of $GABA_A$ receptors. It blocks sustained repetitive firing in cultured neurons (Verdon et al 2000) by state-dependent blockade of voltage-gated Na^+ channels (Nicholson et al 2001). cOA (which can also block gap junctions) may represent an endogenous anticonvulsant. We have previously reported the ability of cOA to suppress spontaneous epileptiform events using the 4-aminopyridine (4AP) model of epilepsy in the CA3 neurons of the in-vitro hippocampal slice (unpublished). Here, we compare carbamazepine and cOA as modulators of evoked epileptiform activity induced by 4AP in CA1 neurons.

Male Wistar rats (150–200 g) were humanely sacrificed and saggital brain slices (400 μm) were prepared for superfusion at 35°C. Field population spikes (fPS) from CA1 stratum pyramidale were recorded following stimulation of the Schaffer collaterals using a bipolar stimulus (100-μs pulses at 0.1 Hz). Artificial cerebrospinal fluid (aCSF) contained 4AP 50 μM and 0.1% DMSO plus 0.1% BSA to aid the dissolution of cOA. No BSA was used in the carbamazepine experiments. We measured the effects on the amplitude of the presynaptic volley (psv), primary (1st), secondary (2nd) and tertiary (3rd) spikes, as well as discharge duration. Results are normalised as % of pre-treatment mean ± s.e.m. Statistical tests are detailed below: $P < 0.05$ was significant.

4-AP evoked protracted epileptiform bursts (control fPS duration, 34.25 ± 1.148ms; after 4-AP, 71.45 ± 3.62 ms). Oleamide (32 μM, 60 min) did not significantly alter the fPS amplitude (control vs cOA 32 μM; 1st 108 ± 9.8 vs 104 ± 4.46, 2nd 109 ± 10.5 vs 103 ± 19.7, 3rd 121 ± 21.8 vs 97 ± 28.3, psv 103 ± 0.65 vs 98 ± 3.304, n = 4, unpaired *t*-test). fPS duration (n = 4, control vs cOA 32 μM, 99.1 ± 6.9 vs 90.25 ± 9.6, respectively, unpaired *t*-test) of the epileptiform events, although a downwards trend was observed. Carbamazepine (100 μM) did not significantly alter amplitude, although an upward trend was observed (n = 5, control vs carbamazepine 100 μM; 1st 102 ± 1.342 vs 113.8 ± 7.1, 2nd 99.2 ± 7.0 vs 126 ± 15.85, 3rd 106.8 ± 18.55 vs 152.8 ± 51.1, psv 109.4 ± 8.2 vs 101 ± 5.1, paired *t*-tests). However, carbamazepine consistently and reversibly reduced the duration of the epileptiform events (n = 5, control vs carbamazepine 100 μM, vs washout, 102.2 ± 3.5 vs 88.6 ± 3.2 vs 101.8 ± 3.2, respectively, $P < 0.001$, analysis of variance/Tukey's post-hoc).

Assuming cOA permeates the slices, it exhibits a different pharmacological profile to carbamazepine, which caused a marginal increase in the amplitude of the events and depressed their duration. cOA does not suppress the interictal-like discharges studied here, but protracted ictal-events, correlating with the appearance of clinical epilepsy, and may respond to the sleep lipid (generally more sensitive to anticonvulsant drugs). Gap junction inhibitors suppress the spread of ictal-like field discharges but do not interfere with primary unitary bursts (e.g. Schweitzer et al 2000). Carbamazepine is a potent blocker in the bicuculline/high-K^+ model in CA1. cOA has yet to be examined in these alternative models.

Nicholson, R., et al (2001) *Anaesthesiology* **94**: 120–128

Schweitzer, et al (2000) *J. Neurophysiol.* 84: 927–933

Verdon, B., et al (2000). *Br. J. Pharmacol.* **129**: 283–290

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231

Comparison of cholinesterase level in pesticide exposed and unexposed volunteers

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The majority of deaths from poisoning in the third world result from cholinesterase-inhibiting pesticides like organophosphates (OPs; Winters et al 1981; Ojanguren et al 1998). The increased pesticide use in Nepal by farmers is of serious health concern (Leela 1995). This study aimed to measure and compare baseline values for pseudocholinesterase (ChE) activity in plasma and acetylcholinesterase (AChE) activity in red blood cells (RBC) of 30 OP-unexposed normal volunteers (NVs) and 30 farmers using OPs. The latter were males who had sprayed OPs at lease once within the 3 months immediately before blood sampling. This test group was selected by face-to-face interview using questionnaire; a consent letter was also obtained from all participants. Blood (3 mL) was collected from each subject by venepuncture and transferred into vacuattes containing 2 mg EDTA. Samples were centrifuged at 3000 rev min^{-1} for 3 min. A centronic test kit developed by Gmbh based on original Ellman's colorimetric method (Henry et al 1974) was used to estimate cholinesterase level in plasma and RBC fraction. Butyrylthiocholine was used as substrate that reacts with cholinesterase and splits into butyrate and thiocholine. The activity of cholinesterase was ascertained from the rate of increase of thiocholine. It reacts with dithiobisbenzoate forming 2-nitro-5-mercapto-benzoate, the colour intensity of which was measured at 436 nm. The data were analysed using SPSS and level of significance was tested by Student's *t*-test at $\alpha = 0.05$.

The observed level of AChE in RBC and ChE in plasma of the unexposed and exposed groups are shown in Table 1.

Table 1 Acetylcholinesterase level in RBC and ChE in plasma

Group	AchE level (U L^{-1}) in RBC	ChE level (U L^{-1}) in plasma
Normal volunteers	340 ± 121	10446 ± 3780
Farmers using OPs	339 ± 111	Not done

Data are expressed as mean ± s.d., n = 30

Cholinesterase activity was lower in the RBC fraction than in plasma of unexposed volunteers. However, due to the large variance of the data, the difference was not significant. While the mean AChE levels were not significantly different between the control and exposed groups, significant differences within the exposed population were found between farmers spraying OPs more than 6 times (221 ± 48 U L^{-1}, n = 10) and less than 6 times (398 ± 82 U L^{-1}, n = 20) and between farmers who did (394 ± 119 U L^{-1}, n = 14) and did not (290 ± 79 U L^{-1}, n = 16) practice safety measures during spraying.

The study demonstrated that in control subjects both AChE and ChE were variable. In all of the farmers using OPs, clinical manifestations similar to those of OP toxicity were observed despite the overall mean AChE being comparable with that of the controls. It is possible that symptoms of OP toxicity do not always correlate with AChE inhibition and may not be the sole cause of severe toxicity (Aron et al 1998).

Aron, C. K., et al (1998) *Goldfrank's toxicologic emergencies*. Appleton and Lange

Henry, R. J., et al (1974) *Clinical chemistry: principles and technique*. Harper U. Row publishers, 917–927

Leela, D. (1995) *Nepal conservation strategy implementation project: a study on pesticide pollution in Nepal*. Nepal Planning Commision

Ojanguren, R. T., et al (1998) *Arch. Environmental Health* **53:** 29–35

Winters, D. R., et al (1981) *Clin. Chem.* **27:** 926–929

232

Activity of nitro derivatives of steroids against histamine-induced bronchoconstriction

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Inhaled nitric oxide (NO) (Hogman et al 1993) and NO donors, such as nitroglycerin (Rolla et al 1995) exert bronchodilator activity in asthmatics. Thus, NO release from NO-derivatives of established asthma drugs may confer beneficial bronchodilator activity. Indeed, we have shown that salbutamol-NO exerts greater bronchodilatation than the parent compound (Toward et al 2001). Here we examine four novel nitro-derivatives of the steroids, prednisone (NCX 1015), budesonide (NCX 1020), flunisolone (NCX 1024) and flumetasone (NCX 1023) as bronchodilators in conscious guinea-pigs.

Male Dunkin-Hartley guinea-pigs (250–300g) were exposed to histamine (3 mM, nose only for 20 s) and again 24 h later. Thirty minutes before the second histamine challenge, guinea-pigs (n = 6) received inhaled (15 min) flumetasone-NO, flunisolone-NO, budesonide-NO (each at 0.6 mg mL^{-1}), prednisone-NO (0.056 and 0.56 mg mL^{-1}), budesonide (0.5 mg mL^{-1}), prednisone (0.373 mg mL^{-1}) or vehicle. The vehicle for prednisone and high-dose prednisone-NO was DMSO–ethanol–saline (20:10:70) and for the remaining compounds in the ratio 30:30:40. Aerosols were generated by a Wright's nebulizer supplied with air (20 lb p.s.i., 0.5 mL min^{-1}), the steroids being delivered into a chamber (30 × 20 × 15 cm). Specific airway conductance (sGaw) of conscious guinea-pigs was measured by whole body plethysmography before (baseline) and at intervals after histamine exposure. sGaw was expressed as the % change from baseline before histamine exposure. Statistical analysis was by paired Student's *t*-test.

Histamine (3 mM) caused bronchoconstriction, the immediate peak fall in sGaw being –27.6 ± 5.2%. When repeated 24 h later, 30 min after inhalation of DMSO–ethanol–saline (30:30:40) vehicle, the response was unaltered (–25.9 ± 4.5%). Similarly, the peak falls in sGaw before (−35.2 ± 7.3%) and after (–39.5 ± 7.6%) DMSO–ethanol–saline (20:10:70) were not significantly different ($P > 0.05$). The bronchoconstriction to histamine was not affected by low dose prednisone-NO (0.056 mg mL^{-1}) (−25.3 ± 6.9, –27.3 ± 9.3%, respectively), but was significantly attenuated by the higher dose (0.56 mg mL^{-1}) (−11.9 ± 1.2, −0.7 ± 3.8%, respectively, $P < 0.05$). Budesonide-NO (−28.1 ± 4.4, −5.1 ± 2.8%, respectively, $P < 0.01$), flumetasone-NO (−25.4 ± 4.7, +0.73 ± 2.3%, respectively, $P < 0.001$) and flunisolone-NO (−25.9 ± 2.8, −0.46 ± 2.9%, respectively, $P < 0.001$) also significantly reduced the bronchoconstriction to histamine. The parent compounds, budesonide −33.4 ± 5.1, −39.6 ± 7.4%, respectively) and prednisone (−31.6 ± 8.9, −28.3 ± 3.9%, respectively), at molar equivalent doses, did not reduce the bronchoconstriction to histamine ($P > 0.05$).

The attenuation of histamine-induced bronchoconstriction by budesonide-NO and prednisone-NO but not the parent compounds suggests that the NO component of these two derivatives exerts a bronchodilator action. The bronchoprotection by flunisolone-NO and flumetasone-NO would also appear to be mediated via NO. Thus, these NO derivatives of steroids may have added utility for asthma therapy from a common bronchodilator action.

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Hogman, M., Frostell, C. G., Arnberg, H., et al. (1993) *Am. Rev. Respir. Dis.* **148**: 1474–1478

Rolla, G., Bucca, C., Brussino, L. (1995) *Chest* **107**: 169–172

Toward, T. J., Lewis-Lakelin, M. B., Burgaud, J. B., et al (2001) *Inflammation Res.* **50** (Suppl. 3): S181

233
Relaxant actions of oestrogen on isolated human umbilical arteries

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The umbilical-placental circulation is important in regulating fetal growth and development. Reduced placental circulation (e.g. in hypertension or pre-eclampsia) can retard growth, reduce birthweight and increase neonatal morbidity with adverse implications for health in adult life (Barker 1992). Oestrogen levels increase considerably during pregnancy and may modulate many of the concomitant cardiovascular changes. In mature women, oestrogens in hormone replacement therapy (HRT) are reported to protect against coronary disease (Stampfer et al 1991). Vasodilatation is thought to be important in this and may involve nitric oxide (NO) release from vascular endothelium (Eseh-Sumbele & McCurrie 2000). This project investigated actions of 17β oestradiol (EST) on human umbilical arteries to determine whether NO release may be involved in oestrogen-induced relaxation of these vessels. The study was approved by the Bradford NHS Trust Ethical Committee.

Umbilical arteries, dissected from cord obtained at spontaneous delivery or caesarian section at term, were cut into 1-cm rings. Paired rings were placed in Krebs' solution containing 1 μM indometacin (37°C, 95% O_2/5% CO_2) under 3 g tension. Stable, sustained contraction was elicited by adding 5HT (3 μM, an EC90 concentration) and non-cumulative relaxation-response curves to EST (5–100 μM) or verapamil (VP, 0.1–10 μM) constructed in one ring. A second ring was incubated with the constitutive nitric oxide synthase (NOS) inhibitor, L-NMMA (1–100 μM) for 30 min before administering 5HT (3 μM); EST or VP was added as before. In further experiments, following pre-contraction by 5HT (3 μM), one ring was equilibrated with L-arginine (L-ARG, 10–100 μM) or the NO donor, sodium nitroprusside (NP, 1–100 μM). The second was contracted by 5HT and treated with L-ARG (10–100 μM) for 10 min before adding EST (10–100 μM). Vehicle (60% alcohol/40% water) produced < 20% relaxation.

5HT (3 μM) induced 2.3 ± 0.2 g tension, sustained for 50–60 min. EST caused concentration-related, slow relaxation, maximum relaxation being 63.0 ± 7.5%; verapamil (10 μM) completely reversed contraction. L-NMMA (10–100 μM) potentiated contractile responses to 5HT, maximum 66.0 ± 4.0%, and completely inhibited EST-induced relaxation, n = 5 (Table1), VP was unaffected. L-ARG (10–100 μM) relaxed 5HT-induced contraction enhancing relaxation elicited by EST (10 μM), n = 7 (Table1). NP (1–100 μM) also relaxed 5HT-induced contraction; maximum relaxation was 83.0 ± 2.3%.

Table 1 Effects of modulating NO on relaxation by EST (10–100 μM) in umbilical rings (% reversal of 5HT-induced contraction)

Oestradiol	% Reversal of contraction	+ L-NMMA (100 μM)	+ L-arginine (100 μM)
10 μM	33.0 ± 5.2	0	52.8 ± 0.8*
50 μM	8.0 ± 7.3	0	59.0 ± 1.5
100 μM	63.5 ± 7.5	0	65.0 ± 3.1

*$P < 0.05$

Oestrogen caused incomplete, slow relaxation of umbilical arteries which was enhanced by the NOS substrate, L-arginine, and abolished by the NOS inhibitor, L-NMMA. This suggests that an NO pathway for relaxation exists in umbilical arteries at term and that NO release contributes to the acute relaxant effects of oestrogen in this tissue.

Barker, D. J. P. (1992) *Mothers, babies and health in later life*. Churchill Livingstone

Eseh-Sumbele, P., McCurrie, J. R. (2000) *J. Pharm. Pharmacol* **152**: 287

Stampfer, M. J., et al (1991) *N. Engl. J. Med.* **325**: 756–762

234
Relaxant effects of oestrogen in blood vessels from aged rats

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Aging involves vascular changes which may progress to coronary or cerebral arterial disease. Beneficial effects of hormone replacement therapy (HRT) in reducing coronary disease and mortality in post-menopausal women have been reported (Henderson & Paganini Hill 1991): the vasodilator actions of oestrogen in HRT appear to be an important component and nitric oxide (NO) production may be involved (Eseh-Sumbele & McCurrie 2000). However, it is not established that vasodilator activity is retained in older age groups. This work compares effects of 17β oestradiol (EST) and sodium nitroprusside (NP, an NO donor) on KCl-induced contraction in blood vessels from adult and aged rats and effects of modifying NO production.

Endothelium-intact aortic rings from male Hooded Lister rats (250–350g) aged 3 months or old, 24-month rats (350–400g), were placed in Krebs' solution containing 10 μM indometacin (37°C, 95% O_2, 5%CO_2) (KS) under 2 g tension. Functional endothelium was confirmed by relaxation (> 30%) to acetylcholine (1 μM) following contraction by KCl (60 mM). Portal veins were studied in KS under 0.5 g tension. Concentration–response curves to KCl (5–80 mM) were constructed before and following incubation with EST (10 μM) or NP (1 μM) in the absence or presence of modulators of NO production or guanylate cyclase inhibition.

Aging did not affect KCl-induced contraction in portal vein or aorta. In 3-month rats, EST (10 μM) reduced contractile responses to KCl in all tissues, shifting concentration–response curves rightwards with reduction in Emax. Relaxation was greater in aged than young animals. NP (1 μM) reduced contractile responses more in aorta than portal vein and this action was greater in aged rats (Table 1). L-NAME (100 μM), which inhibits nitric oxide synthase (NOS), attenuated EST-induced relaxation in aorta but not portal vein. In the presence of L-NAME (100 μM), L-arginine (L-ARG, 100 μM), an NOS substrate, restored relaxation by EST in aorta; portal vein was unaffected (Table 1, *$P < 0.01$, **$P < 0.001$; n = 6).

Table 1 Relaxation (% reversal of maximal KCl-induced response) by EST (10 μM) alone, + L-NAME (100 μM) or + L-NAME + L-ARG (100 μM) in portal vein (PV) and aorta from young and old rats

	Young rats		Old rats	
	Portal vein	Aorta	Portal vein	Aorta
EST	50.6 ± 5.7	51.7 ± 4.6	59.5 ± 2.8	60.5 ± 2.0
NP	19.8 ± 8.0	40.0 ± 6.8	22.0 ± 4.0	75.0 ± 8.3
EST + L-NAME	44.6 ± 8.2	24.1 ± 3.7*	44.3 ± 7.8	0
EST + L-NAME + L-ARG	41.9 ± 8.0	42.7 ± 7.6*	52.1 ± 6.6	67.4 ± 5.0**

In further experiments, following inhibition of nitric oxide-sensitive guanylate cyclase by oxadiazolo-quinoxalin-1-one (ODQ, 1 μM), actions of EST (10 μM) and NP (1 μM) on KCl-induced contraction were retested. ODQ completely inhibited EST-induced relaxation in aorta of both adult and old rats: responses of portal vein were unaffected. ODQ also attenuated NP-induced relaxation in all vessels.

Results show that relaxant effects of oestrogen and NP are maintained in old rats. Relaxation by EST appears to involve NO production and cGMP activity in aorta but not portal vein, where EST relaxed tissues independently of a NO-cGMP pathway. The differences in relaxant mechanisms observed in these two vessels may explain some of the variation in the mechanisms previously suggested to explain the vasodilator actions of oestrogens.

Eseh-Sumbele, P., McCurrie, J. R. (2000) *J. Pharm. Pharmacol.* **52**: 287

Henderson, B., Paganini Hill, A. (1991) *Arch. Intern. Med.* **151**: 75–78

235

Actions of environmental oestrogens on mammalian blood vessels

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Environmental chemicals with oestrogenic properties may act as endocrine disrupters and affect other aspects of animal and human health (Harrison 2001). Xenobiotic oestrogens are derived from plants (phytoestrogens) or industrial sources, the latter include bisphenol-A (BPA), from polycarbonate plastics used in dental resins, linings of food cans and water pipes and nonylphenol (NP), from non-ionic surfactants in detergents. Endogenous oestrogens (e.g. 17β oestradiol (EST)), are 1000–10 000 times more potent and bind more strongly to oestrogen receptors than xenobiotics. Whereas the activity of endogenous oestrogens is short-lived, industrial oestrogens, BPA and NP, are more stable, concentrate in food chains and, being lipophilic, accumulate in human fat. These compounds may antagonise, mimic or modulate actions of endogenous oestrogens with potential reproductive or other effects in human populations. We previously reported dilator actions of phytoestrogens in guinea-pig blood vessels (McCurrie et al 2000). It was therefore of interest to study actions of industrial oestrogens on vascular muscle: we compared BPA and NP and the phytoestrogen, genistein (GEN) with 17β oestradiol (EST) in rat portal vein.

Portal veins from male Hooded Lister rats (200–300 g) were placed under 0.5 g tension in Krebs' solution containing 10 μM indometacin (37°C, 95%O_2, 5%CO_2). Control concentration–response curves to KCl (10–100 mM) were constructed in the absence or presence of EST (2–16 μM), BPA (1–20 μM), NP (1–20 μM) or genistein (GEN, 20 μM). EST, BPA and GEN shifted concentration–response curves rightwards, reducing maximal contraction (Table 1). NP (20 μM) caused a small reduction in KCl-induced contraction, 21.0 ± 7.3%, without shifting the curve.

To investigate possible calcium antagonism of the agents, portal veins were incubated for 1 h in Ca-free Krebs' solution. KCl (30 mM) was added: after 5 min cumulative concentration–response curves to calcium (10 μM–20 mM) were constructed in the absence or presence of EST (2–16 μM), BPA (20 μM), GEN (20 μM), or the calcium channel blocker, nifedipine (NIF, 1–10 nM). NIF (10 nM) shifted concentration–response curves rightwards, reducing Emax by 73.0 ± 5.2%. EST, BPA and GEN also shifted the concentration–response curve to the right, reducing Emax. This reduction by BPA and GEN was not significantly different when contraction was elicited by either KCl or calcium (Table 1).

Table 1 Relaxation (% reversal of maximal KCl or Ca-induced contraction) by endogenous and environmental oestrogens

	KCl	Ca
EST (16 μM)	96.0 ± 2.5	68.5 ± 7.3
GEN (20 μM)	57.0 ± 3.4	59.8 ± 2.3
BPA (20 mM)	64.3 ± 2.5	55.1 ± 4.5

n = 4–7

NP and BPA are both environmental oestrogens but their actions differed: NP caused relatively little relaxation while BPA, at the same concentration, relaxed portal vein in a manner similar to EST and GEN. EST has a 1000-fold higher affinity than BPA for oestrogen receptors (Kuiper et al 1998), however, the apparent relaxant potency of these substances was similar, which suggests that relaxation induced in portal vein does not involve oestrogen receptors. Since actions of EST, GEN and BPA in relaxing Ca-induced contraction resembled the actions of the calcium-channel blocker, NIF, a component of the mechanism by which endogenous and environmental oestrogens relax this tissue may involve calcium-channel blocking activity.

Harrison, P. T. C. (2001) *Br. Med. J.* **323**: 1317–1318

Kuiper, G. G., et al (1998) *Endocrinology* **139**: 4252–4263

McCurrie, J. R., et al (2000) *J. Pharm. Pharmacol.* **52**: 288

236

Acute and chronic exposure to inhaled (1→3)-β-D-glucan causes airway inflammation in the conscious guinea-pig

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(1→3)-β-D-glucan is a polyglucose structure found in the cell wall of moulds, some bacteria and plants. (1→3)-β-D-glucans have been discovered in organic dusts, such as cotton and grain; it is believed that these glucans are associated with the respiratory disease arising from exposure to such dusts (Young et al 1998). Inhalation of (1→3)-β-D-glucan causes symptoms from the upper respiratory tract, namely nose and throat irritation (Rylander 1996).

Male Dunkin-Heartly guinea-pigs (250–300g) were exposed to a single (1→3)-β-D-glucan challenge either on one day (acute study) or on five consecutive days with a two-day rest period, followed by one final exposure on day 8 (chronic study). Guinea-pigs were exposed to nebulised (1→3)-β-D-glucan dissolved in pathogen-free saline (0.9%) or saline vehicle, for one hour in a sealed metal chamber (70 cm diameter, 16 cm deep). The aerosol was generated using a DeVilbiss nebuliser at a pressure of 20 lb p.s.i. at a flow rate of 0.5 mL min^{-1}. One hour after the final glucan exposure, guinea-pigs were killed (by pentabarbitone overdose). The trachea was cannulated and bronchoalveolar lavage (BAL) was carried out (n = 6). The lungs were lavaged with a 1% EDTA solution (1 mL 100g^{-1} body weight) which was passed gently into the lungs using a 5-mL syringe and was recovered 3 min later; this procedure was repeated and the BAL fluid was pooled for each guinea-pig. Total cell counts (TCC) were determined using a Neubauer haemocytometer. BAL fluid (100 μL) was centrifuged (Cytospin II, Shandon) onto glass microscope slides (1000 rev min^{-1} for 7 min). The resulting slides were stained with 1.5% Leishman's stain (in 100% methanol) for 7 min. A minimum of 500 cells was counted and the proportion of macrophages, eosinophils and neutrophils were calculated (Danahay & Broadley 1997). Results are shown in Table 1.

Table 1 Cell population from BAL

	Cell count × 10^6			
	TCC	Macro	Eosino	Neutro
Acute				
Saline	2.4 ± 0.2	2.3 ± 0.27	0.20 ± 0.04	0.00 ± 0.00
30 μg mL^{-1}	2.5 ± 0.4	2.2 ± 0.39	0.30 ± 0.06	0.00 ± 0.00
100 μg mL^{-1}	3.71 ± 0.22*	3.18 ± 0.20	0.40 ± 0.05	0.00 ± 0.00
Chronic				
Saline	1.40 ± 0.07	1.32 ± 0.06	0.10 ± 0.02	0.00 ± 0.00
30 μg mL^{-1}	1.46 ± 0.09	1.290 ± 0.09	0.17 ± 0.03	0.00 ± 0.00
100 μg mL^{-1}	2.28 ± 0.24*	1.92 ± 0.20	0.33 ± 0.07	0.03 ± 0.01

Exposure to (1→3)-β-D-glucan in both the chronic and acute studies produced a significant ($P < 0.05$*) increase (Student's *t*-test) in cell numbers for the 100 μg mL^{-1} dose when compared with control. Although the increase in cell population for the lower glucan dose is not significant it demonstrates that (1→3)-β-D-glucan induced recruitment of inflammatory cells is dose dependent. Increases in the macrophage and eosinophil population was demonstrated in both protocols. However, only chronic exposure led to migration of neutrophils into the lungs.

In this study we have demonstrated that inhalation of (1→3)-β-D-glucan led to inflammatory cell recruitment in the guinea-pig lung, with chronic exposure needed to induce neutrophilia. This phenomenon may be of clinical importance in assessing the respiratory effect of inhaled glucan in man.

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Danahay, H., Broadley, K. J. (1997) *Br. J. Pharmacol.* **120**: 289–297

Rylander, R. (1996) *Indoor Built Environ.* **5**: 106–111

Young, R. S, Jones, A. M., Nicholls, P. J. (1998) *J. Pharm. Pharmacol.* **50**: 11–17

237

Stereoselective and metabolite-mediated antiproliferative effects of non-steroidal anti-inflammatory drugs

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Interest in the anticancer activity of non-steroidal anti-inflammatory drugs (NSAIDs) originated with reports that sulindac can be effective for the treatment of colorectal polyps (Waddell et al 1983). Sulindac is susceptable to oxidative and reductive metabolism in-vivo resulting in the production of sulphone and sulphide metabolites which have reduced and enhanced cyclooxygenase (COX)-inhibiting potency, respectively. Different COX-inhibiting activities are also found in the individual enantiomers of chiral profen NSAIDs, with greater potency generally residing in the (+)-*S*-enantiomers. The aim of this study was to compare the antiproliferative effects of sulindac and its metabolites and also racemic mixtures of five chiral NSAIDs and their individual enantiomers.

The antiproliferative effects of the NSAIDS (Tables 1 and 2) against Caco-2 cells were measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Cells were seeded at 10^4 cells/well in 96-well culture plates and incubated for 18 h under standard culture conditions. The final NSAID concentrations examined were 50, 100, 150, 200, 500, 750, 1000 and 1500 μM in 100 μL serum-free fresh culture medium and 50 μL of NSAID in DMSO (1.8% v/v)/Tris buffer/phosphate-buffered saline (PBS), pH 7.4. After 72 h the MTT assay was performed (Dodhia 2001). The concentration of each NSAID required to inhibit proliferation by 50% (IC50) was calculated using PRISM software.

Table 1 IC50 values (μM) for Caco-2 cell growth inhibition by sulindac and metabolites measured using a MTT assay

NSAID	Description	COX activity	MTT Caco-2 IC50 (μM)
Sulindac	Parent	Weak, non-selective	178 ± 10
Sulindac sulphone	Oxidation product	Inactive	582 ± 13*
Sulindax sulphide	Reduction product	Potent, non selective	115 ± 9*

Data represent mean ± s.d., n = 6 (pooled from 2 × n = 3 measurements per experiment). *$P < 0.05$, vs sulindac, using Tukeys test

Table 2 IC50 values (μM) for Caco-2 cell growth inhibition by racemic mixtures and individual enantiomers of NSAIDs measured using a MTT assay

	IC50 values (μM) for MTT assay in Caco-2 cells		
NSAID	Racemate	(+)-*S*	(−)-*R*
Pirprofen	140 ± 4	137 ± 2	146 ± 6
Carprofen	155 ± 6	107 ± 8*†	202 ± 11*†
Flurbiprofen	249 ± 7	224 ± 16	285 ± 19
Ibuprofen	261 ± 10	208 ± 9*†	753 ± 3*†
Indobufen	718 ± 10	497 ± 3*	nd

Data represent mean ± s.d., n = 6 (pooled from 2 × n = 3 measurements per experiment). *$P < 0.05$, vs racemic mixture (Tukeys test); †$P < 0.05$, (+)-enantiomer vs (−)-enantiomer (Tukeys test,); nd = not determined

The data determined from an examination of sulindac and its metabolites and the individual enantiomers of the profens indicate that COX inhibition is not an essential feature for the antiproliferative effects of these agents. Interestingly, sulindac sulphone, the only agent thus far used in man, showed the least potency compared with sulindac and the sulphide. These data, taken together with that of the individual profen enantiomers, indicate that the non-COX inhibitory (−)-*R*-enantiomers may be potentially useful anticancer agents with reduced side effects.

Dodhia, V. (2001) MSc Thesis, University of London

Waddell, W. R., et al (1983) *J. Surg. Oncol.* **24**: 83–87

238

Antiproliferative effects of non-steroidal anti-inflammatory drugs on Caco-2 cells

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Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to have an inhibitory effect against the proliferation of colorectal cancer cells. However, the molecular basis of NSAID activity and the role of cyclooxygenase (COX) in this action remains unclear. The aim of this study was to investigate the relative potency of the antiproliferative effects of NSAIDs on Caco-2 cells (derived from a colonic adenocarcinoma) and to compare these effects with published values for inhibition of COX.

The antiproliferative effects of fourteen NSAIDs (Table 1) on Caco-2 cell growth were measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Caco-2 cells were seeded at 10^4 cells/well in 96-well culture plates and incubated for 18 h at 37°C in an atmosphere of humidified 5% CO_2 to allow the cells to enter the exponential growth phase. The cell culture medium was Dulbecco's modified Eagle medium with 10% v/v fetal bovine serum, 1% v/v non-essential amino acids, 2 mM L-glutamine and 100 IU penicillin mL^{-1} and 100 μg streptomycin mL^{-1}. After 18 h the cell culture medium was replaced with 100 μL fresh serum-free cell culture medium and 50 μL of NSAID in DMSO (1.8% v/v)/Tris buffer–phosphate-buffered saline (PBS), pH 7.4, and incubated for 72 h. The final NSAID concentrations tested were 50, 100, 150, 200, 500, 750, 1000 and 1500 μM (n = 5). After 72 h the MTT assay was performed (Dodhia 2001). The concentration of each NSAID required to inhibit cell proliferation by 50% (IC50) was calculated using PRISM software to fit the data.

Table 1 IC50 values for Caco-2 growth inhibition by NSAIDs measured using a MTT assay (mean ± s.d., n = 5) compared with literature values for COX inhibition (Warner et al 1999)

NSAID	MTT Caco-2 IC50 (μM)	COX-1* IC50 (μM)	COX-2* IC50 (μM)
Phenylbutazone	129 ± 15	—	—
Carprofen	155 ± 14	0.087	4.3
Butiprofen	200 ± 11	—	—
Ibuprofen	252 ± 12	7.60	7.2
Flurbiprofen	269 ± 13	0.072	5.5
Benoxaprofen	357 ± 14	—	—
Indometacin	381 ± 11	0.013	1.0
Ketoprofen	512 ± 13	0.047	2.9
Indoprofen	559 ± 13	—	—
Naproxen	595 ± 18	9.30	28.0
Indobufen	744 ± 17	—	—
Tiaprofenic acid	811 ± 13	—	—
Aspirin	> 1500	1.7	> 100
Salicyclic acid	> 1500	—	—

Antiproliferative effects on Caco-2 cells allowed IC50 values to be calculated for 12 of the 14 NSAIDs examined. Aspirin and salicylic acid, relatively weak inhibitors of COX-1 and COX-2, had the least effect on cell growth as determined by the MTT method. Comparison of the IC50 values for MTT conversion by Caco-2 cells (Table 1) with inhibition of COX indicated a lack of relationship between the parameters when examined in-vitro. Phenylbutazone was the most potent inhibitor of cell proliferation, but this compound is generally cytotoxic. Both ibuprofen and flurbiprofen were antiproliferative, are relatively non-toxic agents and may be the most promising candidates for future work.

Dodhia, V. (2001) MSc Thesis, University of London

Warner, T. D., et al (1999) *Proc. Natl Acad. Sci. USA* **96**: 7563–7568

239

Calcium/calmodulin-dependent protein kinase II (CaMK II) and tyrosine kinases (TKs) have a beneficial role whereas Ras/GTPase has a detrimental effect on recovery of cardiac function after global ischaemia

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Cardiac ischaemia arising from coronary heart disease or heart surgery can lead to cardiac failure and death. To develop rational therapeutics for this condition, it is important to have a systematic understanding of the underlying intracellular signaling pathways involved. In this study, we have examined the role of Ras-GTPase, CaMK II and tyrosine kinases (TKs) in recovery of cardiac function following induction of ischaemia in a rat perfusion model of global ischaemia.

Male Wister rats pretreated with drugs were sacrificed and hearts removed for mounting on a Langendorff Perfusion Assembly. A water-filled balloon was introduced into the left ventricle and connected to a Statham Pressure Transducer to obtain outputs of cardiac function. Hearts were exposed to a 1-h ischaemic episode (i.e., absence of perfusion). In pre-conditioning experiments, hearts were exposed to three 5-min cycles of ischaemia followed by reperfusion before the 1-h ischaemic episode. Hearts were studied in 5 groups: Group 1, plain ischaemia (I); Group 2, ischaemia and preconditioning; Group 3, ischemia and pretreatment with FPT III; Group 4, ischaemia and pretreatment with KN-93; and Group 5, ischaemia and pretreatment with genistein. FPT III, KN-93 and genistein are inhibitors of Ras/GTPase, CaMKII and TKs, respectively.

Table 1 Mean % post ischaemic recovery of left ventricular contractility and haemodynamics

Group	Pmax	LVEDP	CF	CVR
I	36 ± 9	653 ± 49	36 ± 5	326 ± 96
I+PC	62 ± 8*	296 ± 21*	67 ± 13*	167 ± 38*
I+FPT III	82 ± 3*	334 ± 42*	45 ± 4*	239 ± 24*
I+KN93	16 ± 3*	868 ± 38*	18 ± 3*	764 ± 68*
I+Genistein	13 ± 6*	754 ± 36*	14 ± 3*	846 ± 53*

I = Ischaemia; PC = preconditioning; Pmax = left ventricular developed pressure; LVEP = left ventricular end diastolic pressure; CF = coronary flow; CVR = coronary vascular resistance. *$P < 0.05$, significantly different from ischaemia alone

Table 1 shows that recovery of cardiac function following 1-h ischaemia was only 36% of left ventricular contractility as indicated by Pmax. Similarly, recovery of haemodynamics was only about 36% as indicated by CF. Preconditioning resulted in a marked improvement in Pmax and CF (approx. 80% increase) and a concomitant decrease in LVEDP (indicating an increase in left ventricular contractility) and CVR (indicating an increase in left ventricular haemodynamics). Pretreatment of rats with FPT III enhanced recovery above that observed with PC. However, pretreatment with KN93 or genistein had detrimental effects on recovery from cardiac ischaemia.

The Ras/GTPase signaling pathway appears to have a detrimental role in cardiac ischaemia, whereas TKs and CaMKII play an important role in mediating pathways that lead to recovery from global ischsemia.

240

The effect of cytochrome P450 polymorphism on anticoagulation with warfarin

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Cytochrome P450 isoform 2C9 (CYP2C9) is the principal enzyme in the metabolism of the *S* optical enantiomer of warfarin. The *S* enantiomer is more potent anticoagulant but is more rapidly metabolised than *R*-warfarin. CYP2C9 polymorphism (i.e., presence of mutant 2C9*2 or 2C9*3 alleles) has been suggested to increase sensitivity to warfarin. This study investigated associations between CYP2C9 polymorphism, and steady-state warfarin total and enantiomer plasma levels, and dose requirements in the sample population in a single hospital. The 167 randomly recruited attendees of a hospital-based anticoagulation clinic were genotyped for their CYP2C9 polymorphism using polymerase chain reaction (PCR) and post-PCR restriction enzyme digestion. Warfarin total and warfarin *S*- and *R*-enantiomer plasma levels were measured from three subsequent study-visit blood samples using reverse-phase chiral HPLC. Patients' maintenance doses before each of the three study visits were recorded.

Allele frequencies in the sample were 0.83, 0.11 and 0.06 for 2C9*1, 2C9*2 and 2C9*3 alleles, respectively. Table 1 summarizes means for the tested variables by genotype.

Table 1 Observed tested variables per genotype

	*1*1	*1*2 and *2*2	*1*3 and *3*3
Total warfarin	1.27 ± 0.53	1.24 ± 0.38	1.21 ± 0.41
S-warfarin	0.41 ± 0.21	0.46 ± 0.16	0.51 ± 0.20
R-warfarin	0.86 ± 0.35	0.78 ± 0.24	0.69 ± 0.26
Dose	5.52 ± 2.53	4.62 ± 1.85	3.67 ± 1.45

Data are presented as means ± s.d.

The effect of CYP2C9 polymorphism on the tested variables was explored using general linear modelling with CYP2C9 genotype as the factor and the relevant covariates. CYP2C9 polymorphism had significant effect on the levels of *S*-warfarin that increased from those with 2C9*1*1 to those with 2C9*3 allele ($P < 0.0001$), and on the maintenance warfarin doses that decreased from 2C9*1*1 to 2C9*3 ($P = 0.006$). There was no significant effect of CYP2C9 on the total warfarin ($P = 0.296$) or *R*-warfarin plasma levels ($P = 0.399$).

The data confirmed that CYP2C9 is significantly associated with increased steady-state plasma levels of the more potent *S* enantiomer of warfarin. Consequently, the patients bearing CYP2C9*2 and *3 variants required lower doses of warfarin. This may have important implications for the initiation of warfarin treatment in patients with variant alleles. With the knowledge of their CYP2C9 genotype, their initial warfarin dose may be decided accordingly, preventing possible risks of bleeding from over-coagulation.

241

Modulation of rat striatal dopamine release by agonists of the glycine co-agonist site of the NMDA receptor

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The pathology underlying schizophrenia is believed to involve several neurotransmitter systems. The clinical efficacy of classical antipsychotics strongly correlates to their ability to block dopamine D2 receptors, although administration of dopamine agonists does not reproduce all of the symptoms of schizophrenia. This suggests that mechanisms other than simple overactivity of the dopaminergic system are responsible for schizophrenic symptom production. Studies involving the dissociative anaesthetic ketamine, and the related drug phencyclidine, which block the *N*-methyl-D-aspartate (NMDA) sub-type of the glutamate receptor, have shown that NMDA antagonism produces a collection of symptoms that more closely mimics idiopathic schizophrenia.

It has been proposed that a possible cause of schizophrenic symptoms is a hypo-glutamatergic state that produces a hyper-dopaminergic state. Evidence in support

of the glutamate hypothesis includes the clinical effects of drugs that enhance glutamatergic activity. The NMDA receptor molecule possesses recognition sites for several molecules in addition to NMDA itself. One such site is a co-agonist site for glycine; occupation of this site is a prerequisite for the opening of the ion channel and, thus, activity of the receptor. Heresco-Levy (2000) has shown that adjunctive therapy with glycine site agonists in addition to antipsychotic treatment can produce a significant improvement in psychotic symptoms.

In this study we have investigated the effects in-vitro of glycine site agonists on the basal and NMDA-induced dopamine release from rat striatal preparations. We have used a static release model in which striatal prisms were placed in gauze baskets and suspended in artificial cerebrospinal fluid (aCSF). At 5-min intervals, the aCSF media were changed and replaced with fresh aCSF; at 20 and 40 min after the start of the experiment the slices were stimulated with NMDA (10 μM) for 5 min; drugs being tested were added from 30 min. Samples were assayed for dopamine content using High-Pressure Liquid Chromatography with electrochemical detection.

In control conditions repeated application of NMDA induces reproducible increase in dopamine release. The application of 10 μM of the glycine site agonists D-serine and D-cycloserine to the incubation medium significantly suppressed the NMDA response by 30% and 38%, respectively. Only 1 mM (but not 10 μM) of glycine significantly reduced NMDA-evoked dopamine release. The suppressive effect of D-cycloserine was prevented by the GABA-A antagonist bicuculline (100 μM).

These results indicate that glycine-site agonists can modulate striatal dopamine release. The inhibitory effects of the co-agonists are possibly related to GABAergic transmission since the effect of D-cycloserine was abolished by co-administration of bicuculline.

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Heresco-Levy, U. (2000). *Int. J. Neuropsychopharm.* **3**: 243–258

242
Angiotensin receptor expression in the aorta and ascending colon of normotensive and hypertensive rat strains

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We have previously reported that contractile responses of the isolated aorta, but not the isolated colon, to angiotensin (Ang II) were significantly reduced in hypertensive strains of rat (Bakhit et al 2001). In this study, we investigated whether these differences are a reflection of changes in the level of expression or the ratios of the different angiotensin receptor subtypes in these tissues. Radioligand binding studies were performed on membrane preparations from aorta and ascending colon from spontaneously hypertensive (SHR) and Brattleboro (BB) rats and their normotensive counterparts, Wistar Kyoto (WKY) and Long Evans (LE), respectively. Receptor subtypes were characterised using losartan and PD123 319 as specific Ang II antagonists at the AT_1 and AT_2 receptors, respectively. The results for receptor density (Bmax) are presented in Table 1.

Table 1 Comparison of AT_1 and AT_2 receptor densities (B_{max}; fmol/mg protein) in isolated colon and aorta from normotensive and hypertensive rats

Tissue	WKY	SHR	LE	BB
Colon				
AT_1	197 ± 10	95 ± 10*	410 ± 70	120 ± 10*
AT_2	213 ± 10	92 ± 10*	400 ± 60	ND*
Aorta				
AT_1	122 ± 8	98 ± 2	200 ± 10	90 ± 10*
AT_2	ND	68 ± 3*	70 ± 10	70 ± 4

*$P < 0.001$ vs normotensive counterparts. ND = not detected; n = 6 in all cases

AT_1 receptor expression in both the colon and aorta were seen to be lower in hypertensive strains compared to their normotensive counterparts. These changes were highly significant ($P < 0.001$) between the aorta and colon of the BB and LE strains but were only significant for the colon of the WKY and SHR strains.

Differences in AT_2 receptor expression were more variable. In the colon there was significantly lower AT_2 receptor expression in both hypertensive strains compared to their normotensive counterparts. However, there was significantly greater expression of the AT_2 receptor in the SHR strain compared to the WKY, but no significant difference in expression between the LE and BB rats.

Comparison of the binding study data presented here with the functional data presented in Bakhit et al (2001) showed that the strain differences in the responsiveness of the tissues to Ang II are not simply correlated with either AT_1 or AT_2 receptor levels or the AT_1:AT_2 receptor ratio. We therefore conclude that strain differences in Ang II responsiveness reflect a combination of the changes in the receptor density and post-receptor changes in the transduction system or the contractile function of the muscle.

Bakhit, D., Yeoman, M. S., Gard, P. R., et al (2001) *British Pharmaceutical Conference Science Proceedings*. London, Pharmaceutical Press p. 190

243
Tyrosine kinases (TKs) and cytochrome P-450 (CYP-450) metabolites of arachidonic acid (AA) play a role in development of cardiovascular dysfunction in diabetes

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Diabetes mellitus is a complex metabolic disease caused by impairment of insulin signalling pathways and this disease can cause many chronic complications such as vascular disease, retinopathy, kidney disease, neuropathy and heart disease. It is likely that glucose and its metabolites mediate their adverse effects by altering various signal transduction pathways. Studies have shown that CYP-450 metabolites of AA and TKs play significant roles in development of hypertension and related organ pathologies. However, their roles in development of diabetes and diabetes-related cardiovascular complications are not known. This study examined the role of CYP-450 metabolites of AA and TKs in development of altered vascular sensitivity to vasoactive hormones in diabetic rats.

Four groups of female Wistar rats were used: Group I, control rats were injected with the citrate buffer vehicle; Group II, diabetic rats without treatment — diabetes was induced by a single intraperitoneal (i.p) injection of 55 mg kg^{-1} streptozotocin (STZ); Group III, diabetic rats that received genistein treatment (0.3 mg/200 g, as an inhibitor of TKs); Group IV, diabetic rats that received 50 mg kg^{-1} aminobenzotriazole (ABT; an inhibitor of CYP450). Genistein and ABT treatment were given i.p. every other day during the last 2 weeks of the study. At the end of four weeks, the rats were sacrificed and the mesenteric beds were isolated to measure changes in perfusion pressure in response to noradrenaline (NA) (10, 100 and 1000 nmol). Ring segments of the renal and carotid arteries (4–5 mm) were mounted in organ-baths to measure isometric contractions. The vasoconstrictor effect of noradrenaline (NA; norepinephrine) (10^{-8}, 10^{-7} and 10^{-6} M) was tested. Hyperglycaemia persisted in the treated rats and was 610 ± 24.4 mg dL^{-1} at four weeks as compared with 85.88 ± 5.0 mg dL^{-1} in the control rats. Table 1 shows that induction of diabetes resulted in increased vascular reactivity to NA in all the tissues studied. Genistein treatment normalized vascular reactivity to NA in the mesenteric bed and carotid artery but not in the renal artery whereas ABT treatment normalized vascular reactivity only in the renal artery.

Table 1 NA-induced vasoconstriction in the perfused mesenteric bed and isolated carotid and renal artery segments

Dose of NA	I (Control)	II (Diabetes)	III (Diabetes + Genistein)	IV (Diabetes + ABT)
Mesenteric bed (mmHg)				
10 nmol[$]	39 ± 14	49.7 ± 14.2	30.6 ± 14.9	27.9 ± 5.5
100 nmol[$]	97.5 ± 14.9	160 ± 15*	62.5 ± 8.5**	141.1 ± 20.4
1000 nmol[$]	118.7 ± 8.8	172.5 ± 17.5*	80.0 ± 2.9**	138.1 ± 24.8
Carotid artery (mg/mg tissue wt)				
10^{-8} M	26.0 ± 4.4	54.4 ± 22.9	28.6 ± 15.2	40.7 ± 9.2
10^{-7} M	57.7 ± 5.4	156 ± 22.6*	72.5 ± 12**	122.1 ± 14
10^{-6} M	78.1 ± 5.4	210.2 ± 22.1*	104.9 ± 13.6**	162.5 ± 18.8
Renal artery (mg/mg tissue wt)				
10^{-8} M	11.8 ± 5.8	64.1 ± 16.9*	94.9 ± 42.1	17.2 ± 8
10^{-7} M	74..8 ± 19.3	158.5 ± 30.7*	212.1 ± 62.5	39.5 ± 8.7**
10^{-6} M	97.9 ± 21.7	218.1 ± 39.9*	353.9 ± 51.3	97.9 ± 21.1**

Data are presented as means ± s.e. *Value significantly different from control, **value significantly different from diabetes, $P < 0.05$. $ Dose in nanomoles perfusing the mesenteric bed at a constant perfusion rate of 6 mL min^{-1}

It is concluded that inhibition of tyrosine kinases and cytochrome P-450 metabolites of arachidonic produces differential improvement of cardiovascular reactivity in diabetic rats.

244
Contribution of Ras GTPase/MAP kinase pathway to angiotensin II– and deoxycorticosterone-salt-induced hypertension

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We previously reported that noradrenaline (norepinephrine) and angiotensin II activate the Ras/mitogen activated protein kinase (MAP kinase) pathway through generation of cytochrome P-450 (CYP-450) and lipoxygenase metabolites (Muthalif et al 1998). The purpose of this study was to determine the contribution of Ras/MAP kinase to angiotensin II (Ang II)- and deoxycorticosterone acetate (DOCA)-salt induced hypertension in rats. Administration of Ang II (350 ng min^{-1} for 6 days) or DOCA and 1% saline drinking water to uninephrectomized rats for six weeks significantly elevated mean arterial blood pressure (MABP) (171 ± 3 mmHg and 166 ± 5 mmHg, respectively) as compared with that of normotensive controls (95 ± 5 mmHg,) ($P < 0.05$). The activity of Ras and MAP kinase, measured in the heart by Western blot analysis, was increased in both Ang II- and DOCA-salt hypertensive rats. Infusion of Ras farnesyl transferase inhibitor, FPT III (138 ng min^{-1}) significantly ($P < 0.05$) attenuated MABP to 116 ± 6 mmHg and 139 ± 4 mmHg in Ang II- and DOCA-hypertensive rats, respectively. Moreover, infusion of MAP kinase kinase inhibitor PD-98059 (694 ng min^{-1}) also reduced MABP to 120 ± 6 mmHg and 126 ± 4 mmHg in Ang II- and DOCA-hypertensive animals, respectively. Morphological studies of the kidney showed that treatment of rat with FPT III which reduced Ras activity minimized the hyperplastic occlusive arteriosclerosis and fibrinoid vasculitis observed in untreated hypertensive rats. These data suggest that the Ras/MAP kinase pathway contributes to Ang II- and DOCA-salt-induced hypertension and associated vascular pathology.

Muthalif, et al (1998) *Proc. Natl Acad. Sci. USA* **95**: 12701–12706

245
Actions of insulin on mammalian aorta

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Epidemiological studies have demonstrated associations between metabolic diseases such as obesity and diabetes and cardiovascular disease such as hypertension, a common feature being insulin resistance and endothelial dysfunction. Hyperinsulinaemia has been reported to be a risk factor for hypertension (Kimura et al 2002). Acute administration of insulin causes vasodilatation that has been attributed to endothelium-derived nitric oxide (NO) release by Scherrer & Sartori (2000) but reported to be independent of endothelium and NO by Izhar et al (2000). In these experiments we studied the actions of physiological and pharmacological concentrations of insulin on KCl-induced contraction in intact and endothelium-denuded (ED) rat aorta.

Endothelium-intact or denuded aortic rings, prepared from Hooded Lister Rats (250–300 g) were placed under 2 g tension in Krebs' solution containing 10 μM indometacin (37°C, 95% O_2, 5% CO_2). Functional endothelium was confirmed in intact rings by relaxation (> 30%) to acetylcholine (1 μM) following contraction by KCl (60 mM). Concentration–response curves were constructed to KCl alone and in the presence of bovine insulin (INS, 0.1, 1.0, 10.0 mU mL^{-1}), the non-selective nitric oxide synthase (NOS) inhibitor L-NAME (100 μM) or L-iminoethyl-lysine (L-NIL, 100 μ) a selective inhibitor of inducible NOS (iNOS). Actions of insulin (0.1, 1.0, 10m U mL^{-1}) were retested following incubation of rings with L-NAME (100 μM) or L-NIL (100 μM) for 20 min, n = 4.

In intact rings, INS 0.1, 1.0 and 10.0 mU mL^{-1} reduced KCl-induced contraction and decreased Emax by 8.8 ± 1.7, 10.2 ± 0.4 and 19.7 ± 5.6%, respectively ($p < 0.01$). However, in ED rings, INS 0.1, 1.0 and 10.0 mU mL^{-1} enhanced contractile responses to KCl: Emax increased by 15.9 ± 2.6, 25.3 ± 3.3 and 46.1 ± 3.4%, respectively ($P < 0.01$).

Both L-NAME and L-NIL (100 μM) enhanced KCl-induced contraction in intact rings: Emax increased by 26.0 ± 2.8 and 44.2 ± 3.0%, respectively ($P < 0.05$). However, in ED rings L-NAME was ineffective, L-NIL (100 μM) enhanced maximal KCl-induced contraction by 30.6 ± 4.9% ($P < 0.05$).

Relaxation of contraction by insulin in intact aorta was prevented by both L-NAME and L-NIL. In ED rings L-NAME (100 μM) was without effect on the actions of insulin but L-NIL (100 μM) further increased the potentiation of contractile activity elicited by the highest concentration of insulin (10 mU mL^{-1}) by 17.0 ± 3.2% ($P < 0.05$).

These results confirm that the endothelium modulates the actions of insulin in rat aorta. In the presence of endothelium insulin is a relaxant, in its absence insulin enhances contractile responses. The relaxant action of insulin appears to depend on NO synthesis since relaxation was prevented by the NOS inhibitors, L-NAME and L-NIL. L-NIL is approximately 30 fold more selective for iNOS than L-NAME. L-NIL increased the potentiation of KCl-induced contraction by insulin (10 mU mL^{-1}) in de-endothelialised aorta, suggesting that vascular muscle may be able to synthesise NO via iNOS, even in the absence of endothelial cells.

The mechanism underlying the potentiation of contraction by insulin in the absence of the endothelium is unknown, but this observation raises the possibility that when NO production is diminished by endothelial damage via atherosclerosis or other factors, circulating insulin may cause vasoconstriction and so exacerbate the development of hypertension.

Izhar, U., et al (2000) *Coronary Artery Dis.* **11**: 69–76
Kimura, M., et al (2002) *Hypertension* **39**: 35–40
Scherrer, U., Sartori, C. (2000) *Eur. J. Endocrinol.* **142**: 315–323