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Shifts in metabolic parameters surrounding glucose homoeostasis resulting from tricyclic antidepressant therapy: implications of insulin resistance?

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Abstract

This study displayed the physiological effects the tricyclic antidepressants amitriptyline or trimipramine have on glucose homoeostasis in male Wistar rats. An insulin secreting cell line (INS-1) was also used to determine effects tricyclic antidepressants have on insulin secretion and insulin displacement. Thirty rats each received a 1 mg kg^{-1} dose of amitriptyline or trimipramine for a period of 14 weeks; another 14 rats served as the control group. Blood glucose, serum insulin and muscle and liver glycogen levels were determined. Kidney, liver and muscle insulin degradation was measured and compared with insulin degrading enzyme concentrations in the latter two tissues. INS-1 cells were used to determine the effect 1 μM amitriptyline has on insulin secretion. Displacement studies for [^{3}H]glibenclamide by amitriptyline or trimipramine were undertaken on INS-1 cells. A significant increase in blood glucose ($P < 0.01$) was found for both test groups after 6 and 14 weeks of receiving the medication, which may be related to a significant decrease in liver and muscle glycogen levels ($P < 0.001$). Serum insulin levels remained unchanged, although a significant increase in insulin degradation was observed in the muscle, liver and kidney, which may be related to a significant increase in insulin degrading enzyme ($P < 0.001$) that was found. A significant increase in insulin secretion was observed for the INS-1 cells treated with amitriptyline, although no significant displacement for the [^{3}H]glibenclamide was evident for amitriptyline or trimipramine. The significant alterations in glucose homoeostasis observed, as well as the significant changes associated with insulin secretion and degradation associated with amitriptyline or trimipramine treatment, imply that prolonged use of these medicines may lead to insulin resistance and full blown diabetes.

Introduction

Data from the Baltimore site of the Epidemiologic Catchment area program found the risk of developing type II diabetes over a period of 12 years to be twice as high in patients already suffering from depression (Eaton et al 1996; Kawakami et al 1999). However, no question was raised as to whether this statistic was linked to the type of antidepressant medication being administered to these patients or whether the depression was itself the root of the diabetes that followed. A series of unrelated case reports confirmed that the antidepressant medication administered to three patients was, in fact, linked to the development of insulin resistance. These patients received serotonin reuptake inhibitor therapy for their depression and as a result displayed a decreased glucose tolerance with an increased insulin secretion, accompanied by increased insulin resistance, after receiving an oral glucose tolerance test. The circulating serum insulin levels were significantly reduced from 324 to 182 μU mL^{-1}, 570 to 306 μU mL^{-1} and 389 to 240 μU mL^{-1} in these three patients after another oral glucose tolerance test was taken (Okamura et al 1999). A recent study also demonstrated how mirtazapine, a tetracyclic antidepressant, improved glucose tolerance, as well as decreased serum insulin and cortisol levels, in eleven clinically depressed patients (Himmerich et al 2006). The treatment lasted approximately six weeks and all the patients displayed improved glucose metabolism despite weight gain being a side effect.

Insulin sensitivity may be related to the catecholamine and serotonin pathways, both of which are affected in clinically depressed patients. Catecholamines, such as dopamine and

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noradrenaline (norepinephrine), have been shown to decrease insulin release and promote insulin resistance, leading to hyperglycaemia (Goodnick 2001). Studies have confirmed that L-dopa and dopamine cause hyperglycaemia at the same time that noradrenaline, which is enzymatically converted from dopamine, promotes insulin resistance (Hakanson et al 1967; Aleyassine & Lee 1972). When catecholamine release is blocked, insulin release and glucose uptake are normalized (Feldman 1976; Lekas et al 1999). These results imply that a prolonged tricyclic antidepressant therapy could ultimately lead to an insulin resistant type II diabetic state in the patient. On the other hand, increased serotonin levels have been found to be associated with increasing insulin sensitivity, thereby promoting glucose disposal, as well as reducing serum HbA1c and free fatty acids levels (Willey et al 1994; Greco et al 1995). However, a study undertaken by Gilles et al (2005) described how ketanserin, a $5HT_2$ serotonin antagonist, significantly impaired insulin sensitivity in ten healthy male subjects.

Alterations in the biochemistry of noradrenaline and serotonin lead to reduced and elevated efficiency of glucose utilization, respectively. The tricyclic antidepressants that affect both serotonin and noradrenaline have proven to be most effective in alleviating diabetic neuropathy (Goodnick 2001).

Tricyclic antidepressant therapy has been limited in recent years due to the emergence of selective serotonin reuptake inhibitors (SSRIs), but despite this they are not completely obsolete and are often still prescribed by physicians. Many physicians prefer to prescribe SSRIs to their patients, as they seem to be more effective and generally cause fewer side effects than tricyclic antidepressants (Gallo 1999; Tan 1999). Despite the popular use of SSRIs, tricyclic antidepressants are still used as an effective medication in certain cases, as SSRIs are often associated with increased anxiety. Tricyclic antidepressants may also be used in the case of treatment failure or adverse side effects associated with SSRIs (Gallo 1999).

The cost of tricyclic antidepressants is somewhat lower than SSRIs (Gallo 1999), which appeals to the lower-income class and the elderly, and they are also effective in controlling the pain arising in post-therapeutic neuralgia and peripheral nerve injuries (Nowak & Handford 1999). Long term, chronic amitriptyline treatment produces a number of actions that are uniquely expressed following chronic administration paradigms, and many of these could also influence the neuroplastic changes that occur with neuropathic pain (Esser et al 2003).

The aim of this study is to determine the effect that two commonly utilized tricyclic antidepressants, amitriptyline and trimipramine, have on glucose homoeostasis. The study gives insight into potential risks associated with tricyclic antidepressant therapy.

Materials and Methods

Animals and treatment

This study was approved by the Animal Ethics Committee of the Nelson Mandela Metropolitan University. Forty-four male Wistar rats, 15 weeks of age, with an average weight of 350 g, were received from the animal unit of the Medical Research Council. The rats were randomly divided into two groups of 14 and 30, respectively. The former group served as the control receiving no medication, the latter served as test subjects, with one group of 15, receiving $1\,mg\,kg^{-1}$ amitriptyline and the other 15 rats receiving $1\,mg\,kg^{-1}$ trimipramine in their drinking water. All rats received dog pellets, freely available, and medication was administered on a daily basis. Food and fluid consumption was also monitored on a daily basis. Rat weight was monitored weekly. All rats were housed in an environmental room with controlled temperature ($22 \pm 1°C$) with a 12-h light–dark cycle (0800–2000 h). Principles of laboratory animal care were conducted in accordance with the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and the Animal Ethics Committee of the Nelson Mandela Metropolitan University.

Glucose clearance

To determine the rate of glucose clearance (after 14 weeks treatment), five rats from each group were anaesthetized with an intramuscular injection of ketamine at a dose of $3\,\mu L\,g^{-1}$ after a 12-h starvation period. The femoral vein was exposed and basal blood glucose was determined using an Optium MediSense glucometer. One milliliter of 0.9% (m/v) saline, containing $0.4\,g\,kg^{-1}$ glucose and $1\,\mu Ci/500\,g$ 2-deoxy-D-[2,6-^{3}H]glucose ($53\,Ci\,mmol^{-1}$ specific activity; Amersham Bioscience) was injected over 1 min into the femoral vein. Blood glucose was measured with the glucometer every 5 min, thereafter, for 1 h. Glucose clearance was calculated from the area under the resulting progress curves.

Glucose uptake in anaesthetized rats

To determine glucose taken up by the different tissue, the rats used in the glucose clearance experiment were immediately euthanased, after the hour incubation time, by injecting a further dose of ketamine straight into the heart. The liver, testicular fat pads, kidneys and a portion of the hind limb muscle were immediately removed and weighed. The organs were finely chopped and 1 g of each was degraded by boiling in 1 mL of 30% (m/v) KOH. One-hundred microlitres of each extract was then counted for 15 min in 3-mL Packard Ultima Gold scintillation cocktail in a Packard Tri-carb 2300TR liquid scintillation analyser.

Serum glucose and insulin content

The experiments that follow utilized the remaining rats not employed for the glucose uptake studies. Following an intramuscular injection of ketamine at $3\,\mu L\,g^{-1}$, blood was removed from the heart using a 5-mL syringe with a 22-gauge needle and blood glucose was determined with a glucometer. The remaining blood was centrifuged at 2000 *g* at 4°C for 20 min. Serum was collected and insulin levels determined using a radioimmunoassay kit for rat insulin (Linco).

Tissue glycogen content

Liver and hind-limb muscle samples were removed and glycogen content was determined through an adaptation of a

previous method (Suzuki et al 2002). Liver or muscle tissue (0.5 g) was degraded by boiling in 0.5 mL 30% KOH (m/v) for 5 min. Glycogen was precipitated with 1.2 mL ethanol and 0.06 mL saturated Na_2SO_4, followed by centrifugation at 1900 *g* for 20 min in Eppendorf tubes. To each pellet, 0.3 mL of 1.2 M HCl was added and samples were boiled for 2 h. The tubes were cooled and a drop of phenol red indicator was added to allow for neutralization with 0.5 M NaOH. Glucose content was determined using a glucose oxidase kit (Glu-cinet Technicon RA-100 system).

Insulin degradation

Insulin degradation was followed using an adaptation of previous methods (Duckworth 1979; Powers et al 1980). Briefly, 10 μCi of [^{125}I] insulin labelled at TyrA^{14} was reconstituted in 100 μL distilled water, according to manufacturers instructions (AEC-Amersham) to yield an insulin concentration of 5×10^{-8} M. Two-and-a-half microlitres of the diluted [^{125}I] insulin was then mixed with 622.5 μL of DMEM supplemented with 0.5% bovine serum albumin (BSA), to produce the binding buffer. All solutions that were to come in contact with the sample tissue were pre-warmed to 37°C.

Insulin degradation studies were performed for both the 6- and 14-week sacrifice times. Liver was the only tissue tested after the 4-week sacrifice, while liver, kidney and muscle were tested after the 14-week sacrifice. On both occasions the tissue was immediately removed post mortem and washed in PBSA warmed to 37°C. The wash ensured the removal of any excess blood. The tissues were finely sliced, using a scalpel, and again washed with PBSA. Excess fluid was drained from the tissue and 2.5 g of each sample was weighed out and incubated with 1000 μL of the pre-mixed binding buffer solution. Control tubes contained no tissue.

The samples were incubated at 37°C and 50 μL volumes of the binding buffer were removed at 5, 10, 15, 20 and 30 min. These volumes were immediately added to 100 μL of cold 10% trichloroacetic acid (TCA), vortexed and kept on ice for a further 5 min, thereby ensuring the completion of the reaction and precipitation of undegraded insulin. The samples were then microfuged for 5 min before 80 μL of the supernatant was added to a pony vial containing 3 mL Packard Ultima Gold scintillation fluid. Each of the samples was counted for 15 min in a Packard Tri-carb 2300TR liquid scintillation analyser.

Insulin degrading enzyme quantification

Two-hundred milligrams of previously frozen tissue was homogenized, using a Retsch mixer mill type 301, in 1 mL homogenizing buffer containing: 50 mM HEPES, 10% glycerol, 1% Triton X-100 and 0.05 mL g^{-1} protease inhibitor fluid, pH 7.5. Samples were then centrifuged at 10 000 *g* for 10 min at 4°C, and the resultant pellet was discarded. For muscle and liver samples, respectively, 30 and 50 μg total protein were separated on a 7.5% SDS-PAGE gel before being transferred to nitrocellulose membranes for 2 h. The membranes were blocked in 5% BSA overnight at 4°C before being subjected to a 2-h incubation, in 5 mL (1:5000) primary antibody (rabbit anti-insulin degrading enzyme (116 kDa-polyclonal antibody; Merck Chemicals PC730), in accordance with the pre-mentioned relevant dilutions. The membranes were then incubated with a secondary antibody (ECL anti-rabbit IgG, peroxidase-linked species-specific whole antibody (from donkey); Amersham NA934) in a 1:2000 dilution for 2 h before being detected through autoradiography using ECL hyperfilm (Amersham, RPN2103K). The bands were then quantified using the Alpha Innotech, AlphaImager 3400 gel dock system.

Insulin secretion by INS-1 cells

Optimal seeding density was determined as 30 000 cells/well. Cells were seeded into 96-well microtitre plates and cultured for 2 days in complete medium. For the experiments, each well was washed once with 50 μL Krebs-Ringer-bicarbonate-HEPES buffer (KRBH) composed of 118.4 mM NaCl, 4.75 mM KCl, 1.192 mM $MgSO_4$, 2.54 mM $CaCl_2$, 10 mM HEPES, 2 mM $NaHCO_3$, 0.1% BSA [18]. The cells were then exposed to 50 μL preincubation medium (KRBH) for 30 min and, after removal, treatment incubation medium (KRBH including applicable medication) (100 μL/well) was added for 10 min. After this incubation was complete, 50 μL incubation medium was removed and a 50× dilution was made for insulin determination using the LINCO Research rat insulin radioimmunoassay (RIA) kit (Cat No. RI-13K), which makes use of rat insulin as a standard. All incubations were carried out at 37°C. Samples were stored at −20°C until required for insulin determination. Assay buffer from the LINCO kit was used as the diluent for all the dilutions required in these experiments.

INS-I cells were exposed to a positive control: glibenclamide (a sulfonylurea) (1 μM) and the antidepressant, amitriptyline (1 μM). Glucose-free medium acted as the basal-state for insulin secretion.

Binding studies with INS-1 rat pancreatic cells

INS-1 cells were seeded into 24-well plates and once confluency was reached, experimentation was carried out. For total binding wells, 100 μL RPMI 1640 was added, and 100 μL RPMI 1640/unlabelled glibenclamide solution was added to each non-specific binding well. The incubation, carried out at 37°C, was initiated through the addition of 50 μL of [^{3}H]glibenclamide (50 Ci mmol^{-1}) in RPMI 1640 to each well. Once incubation with [^{3}H]glibenclamide was complete, the medium was removed and each well rapidly washed three times with 1 mL ice cold PBS. Fifty microlitres of 1 mM NaOH was added to each well to aid in detachment of the cells from the well surface. Each sample was then added to a scintillation vial containing 3 mL scintillation fluid (Packard BioScience). Each well was rinsed with 50 μL PBS and that was also transferred to the scintillation vials. The vials were shaken and radioactivity was determined using a Packard Liquid Scintillation Analyzer, Tri-carb 2300TR (Packard Instrument Company).

Non-specific binding was determined by incubations in the additional presence of unlabelled glibenclamide in excess (10–100× more than labelled). Specific binding was determined by subtracting non-specific from total binding.

Displacement study inhibition of [^{3}H]glibenclamide binding to INS-1 cells was studied in the presence of [^{3}H]glibenclamide (8 nM) and unlabelled glibenclamide (1.34 nM to 1.34 μM) or the two antidepressants, amitriptyline and trimipramine (0.67–6.67 μM). The concentration of the two antidepressants was in accordance with reported plasma levels. Displacement studies were performed at a temperature of 37°C for 30 min using a [^{3}H]glibenclamide concentration of 8 nM.

Computational study

Molecular modelling was carried out with the molecular modelling and simulation computer program, Hyperchem version 5.1 Pro Hypercube Inc. Once a structure was created, molecular mechanisms and molecular dynamics were utilized to investigate the minimum energy values. Bond-rotary searches were used to calculate rotational energy barriers. Molecular dynamics were performed at 1000 K and time steps of 1 fs to search through the different conformations visited during the dynamics simulation. The method involved the following steps: firstly, minimizing the initial structure to relieve any overlay strained coordinates using a minimization of steepest descent method for 100 cycles until the maximum derivative was less than 0.1 kcal/mol/A; secondly, performing molecular dynamics sampling of conformational space using the following protocol with time steps of 1 fs (heating up to high temperatures and equilibrating at the given temperature (1000 K) for 1 ps and then simulating at this temperature for 300 ps with atomic coordinate trajectories recorded every 1 ps); and, thirdly, retrieving the 300 frames recorded as a history file during the dynamics run and minimizing them as an archive file with a two-step minimization, steepest descent method for the first 100 iterations and then a conjugate gradient method until the maximum derivative was less than 0.001 kcal/mol/A (Janjic et al 1999).

Statistical analysis

Data are expressed as mean ± s.d. or mean ± s.e.m. Results of each experimental group were compared with those of the control group using the unpaired Student's *t*-test for several independent observations. $P<0.05$ was considered significant. For glucose clearance curves, the area under the curve was calculated using GraphPad Prism 3.0. The actual K_D value for [^{3}H]glibenclamide was calculated using GraphPad Prism Version 4.

Results

No significant weight gain or change in food and fluid consumption was shown by either test group, in comparison with the control group, throughout the experiment (results not shown).

Blood glucose

The blood glucose values indicated that both test groups displayed a slight but significant increase in blood glucose levels after both sacrifice periods. At the 6-week sacrifice both amitriptyline and trimipramine displayed a significant ($P<0.01$) increase in blood glucose (5.1 ± 0.37 mM and 5.4 ± 0.38 mM, respectively), in comparison with the control group (4 ± 0.71 mM). At the 14-week sacrifice the amitriptyline group's and the control group's blood glucose remained relatively unchanged from that of the previous sacrifice (5.5 ± 0.81 mM ($P<0.05$) and 4.5 ± 0.49 mM, respectively), while that of the trimipramine group was slightly elevated at 7.2 ± 0.72 mM ($P<0.01$). These values are not dangerously high but seem to be on the rise, particularly for the trimipramine group. The hyperglycaemia may continue if the treatment or dosage is increased, leading to diabetes-associated complications.

Serum insulin

Serum insulin levels remained unchanged for the amitriptyline group (91.1 ± 11.4 pmol L^{-1}), compared with the control group (102.2 ± 14.9 pmol L^{-1}) at the end of the 14-week experiment. The circulating insulin levels seemed to be insignificantly less than that of the control group despite the increased glucose levels displayed for this group.

Tissue glycogen content

The glycogen content in both the muscle and liver were significantly reduced for the test groups, over both sacrifice times, relative to that of the lean control groups (Figure 1). The glycogen content for the trimipramine group was found to be significantly reduced in comparison with that found within the amitriptyline group for the muscle, following the 6-week sacrifice period, and both the muscle and liver, following the 14-week sacrifice period ($P<0.001$).

Insulin degradation

Table 1 shows the percent insulin degraded within the relevant tissues over a 30-min incubation period. A significant increase in insulin degradation was evident for both test groups for the liver, kidney and muscle tissue following the 14-week experiment.

Insulin degrading enzyme quantification

A significant increase in insulin degrading enzyme concentration was noted in both the liver and muscle samples of the trimipramine and amitriptyline groups (Figure 2). This increase in concentration may be the reason for the accelerated rate of insulin degradation found following antidepressant treatment.

Glucose clearance and uptake in anaesthetized rats

No significant change in glucose clearance was found for either test group relative to the lean control (results not shown). No significant difference in 2-deoxy-D-[2,6-^{3}H]glucose distribution was found between the liver, muscle or epididymal fat pads of either test groups and the control group. A significant decrease ($P<0.001$) of 61% and 66%

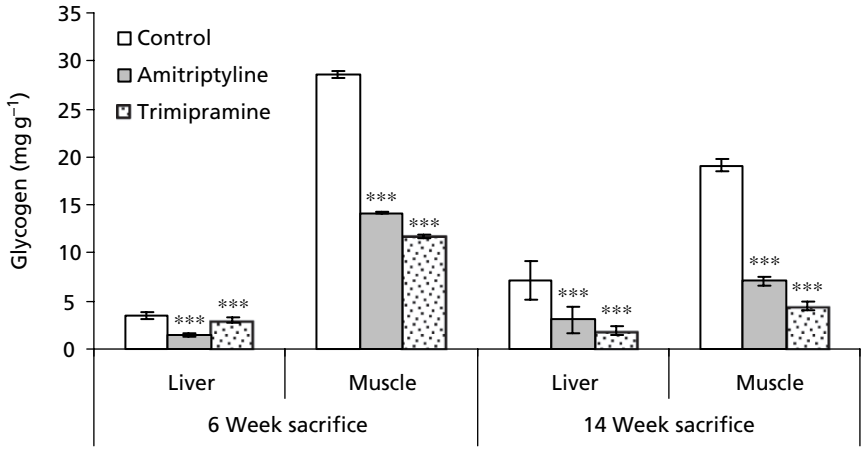

Figure 1 Effects of amitriptyline and trimipramine on tissue glycogen levels in rats. Values are expressed as mean ± s.d., n = 5 rats/group. ***$P < 0.001$ compared with control group.

Table 1 Percentage [^{125}I]insulin degraded by rat liver, kidney or muscle following 14 weeks of treatment with amitriptyline or trimipramine

Time (min)	Control			Amitriptyline			Trimipramine		
	Liver	Kidney	Muscle	Liver	Kidney	Muscle	Liver	Kidney	Muscle
5	32.0 ± 5.9	16.6 ± 2.7	11.0 ± 2.8	18.5 ± 3.5**	11.8 ± 3.5	16.3 ± 7.4	38.2 ± 1.4	19.7 ± 3.3	22.8 ± 2.9†
10	38.9 ± 3.8	32.9 ± 1.2	18.9 ± 1.7	40.2 ± 1.8	34.6 ± 3.7	34.3 ± 2.5***	43.0 ± 1.9	38.8 ± 3.6**	30.7 ± 6.1***
15	37.9 ± 2.0	38.8 ± 2.2	26.6 ± 2.3	43.3 ± 1.8†	48.2 ± 1.8***	36.9 ± 2.5***	42.9 ± 2.5†	44.2 ± 3.1†	34.0 ± 1.1†
20	37.4 ± 1.51	41.7 ± 3.3	28.4 ± 1.5	42.9 ± 1.9**	48.7 ± 3.2†	35.5 ± 0.9	42.8 ± 1.4**	47.1 ± 2.3	34.4 ± 2.1***
30	35.1 ± 1.0	41.0 ± 1.4	30.7 ± 2.0	41.6 ± 2.2†	49.0 ± 3.5†	35.1 ± 2.1*	45.1 ± 2.2***	45.5 ± 2.3†	35.0 ± 1.9**

*$P < 0.05$, **$P < 0.01$, †$P < 0.005$, ***$P < 0.001$ vs control.

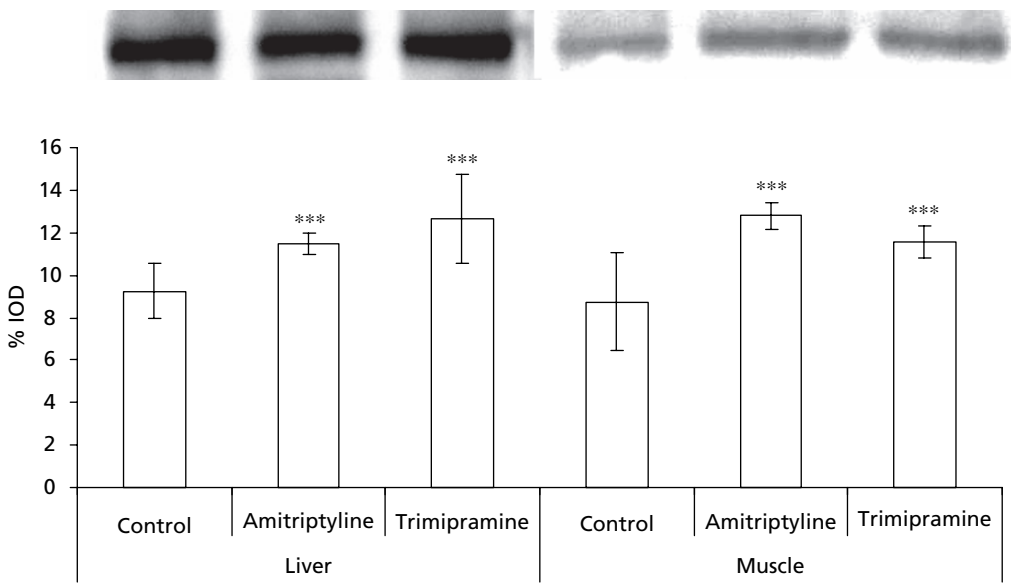

Figure 2 Band density of muscle and liver insulin degrading enzyme in rats after 14 weeks treatment as a percentage of integrated optical density (IOD) obtained from Western blots. Values are expressed as mean ± s.d., n = 5 rats/group. ***$P < 0.001$ compared with control group.

2-deoxy-D-[2,6-^{3}H]glucose was found for the amitriptyline and trimipramine groups, respectively, within the kidney.

Insulin secretion by INS-1 cells

Serum insulin levels of rats were normal, though significant insulin degradation was found in liver, muscle and kidney tissue for both antidepressant groups. These results were confirmed by a significant increase in the presence of the insulin degrading enzyme. To determine whether the normal serum insulin level was the homoeostatic balance between higher insulin secretion coupled with an increase in insulin degradation, the effect of amitriptyline and trimipramine was determined on INS-1 cells. Glibenclamide, the sulfonylurea used by type II diabetic patients, was used as the positive control. Sulfonylureas inhibit K_{ATP}-channels in pancreatic β-cell plasma membranes and thereby initiate insulin release. The released insulin caused by the interaction of sulfonylureas with their receptors is largely preformed insulin because these agents have little immediate effect on insulin synthesis (Foye et al 1995). Insulin secretion was investigated through experiments under acute (10-min exposure) conditions.

Figure 3 shows the stimulation of insulin secretion by exposure to glibenclamide and amitriptyline within acute conditions. Glibenclamide (1μM) produced a significant increase in the amount of insulin secreted compared with the basal control ($P<0.05$) under acute exposure, as expected. In the experiment, amitriptyline (1μM) also stimulated the INS-1 cells to produce significantly more insulin than the basal control ($P<0.05$). In chronic conditions, glibenclamide is not expected to have an effect on insulin secretion due to its transient activity.

Binding studies using INS-1 rat pancreatic cells

Binding experiments were performed to measure the displacement of [^{3}H]glibenclamide (50 Ci mmol^{-1}) by amitriptyline and trimipramine from SUR-1 receptors present in INS-1 rat pancreatic cells. Glibenclamide is a sulfonylurea drug that is used by diabetics and has been reported to bind with high affinity to SUR-1.

For displacement studies, a constant amount of [^{3}H]glibenclamide was added to each of the wells together with varying concentrations of unlabelled competitors of interest. In all the displacement studies carried out, unlabelled glibenclamide was shown to be a significant competitor to [^{3}H]glibenclamide. Figure 4 shows that as the concentration of unlabelled glibenclamide increases, so the percent bound [^{3}H]glibenclamide decreases, indicating displacement of the radioligand.

Figure 4 also reveals that there was no significant displacement of [^{3}H]glibenclamide by amitriptyline or trimipramine. It is evident that there is a large difference between amitriptyline and trimipramine, which could explain the results found in the computational overlaying experiments that identified a 40% chance of binding for trimipramine compared with a 0% chance for amitriptyline.

Computational study

The purpose of carrying out displacement studies was to determine if amitriptyline and trimipramine bound to SUR-1 receptors thereby stimulating insulin secretion; the hypothesis to be tested followed a computational overlay study. By determining the similarity of the sulfonylurea and antidepressants 3-D structures, the possibility of the antidepressant binding to SUR-1 receptors, blocking the K^+_{ATP} channel and resulting in increased insulin secretion was explored. Through the overlay study the probability (P) of a specific antidepressant agent binding to SUR-1 and thereby initiating insulin secretion was determined. For the purpose of the current investigation, two tricyclic antidepressants, amitriptyline and trimipramine, were focused on.

P is the probability of a specific antidepressant agent binding to SUR-1 and thereby initiating insulin secretion. If $P<0.05$, this indicated that the Root Mean Square (RMS) of a particular antidepressant was significantly different to the training set's selected RMS range and there was therefore no possibility of binding. However, if $P>0.05$ there was a 40% chance that binding would occur. The P value calculated for amitriptyline ($P=0.024$) revealed that there would be no

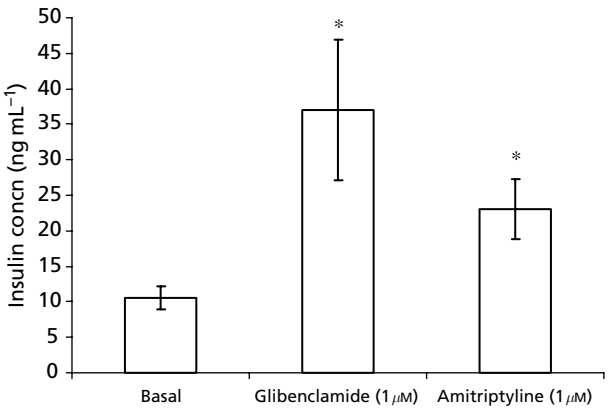

Figure 3 Effect of glibenclamide and amitriptyline on insulin secretion in INS-1 rat pancreatic cells under acute conditions (10 min exposure). Values are expressed as mean ± s.e.m. from an individual experiment assayed in quadruplicate. The experiment was performed three times with similar results. *$P<0.05$ compared with basal.

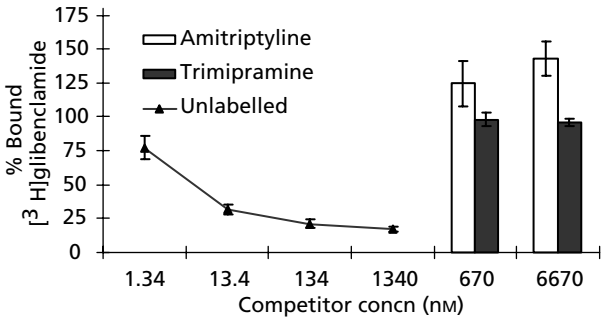

Figure 4 Displacement of [^{3}H]glibenclamide by unlabelled glibenclamide (1.34nM to 1.34μM), amitriptyline and trimipramine (0.67 μM and 6.67 μM, respectively) from SUR-1 receptors in INS-1 rat pancreatic cells. Values are expressed as mean ± s.e.m. from an individual experiment assayed in quadruplicate.

possibility of binding to SUR-1, whereas trimipramine ($P = 0.49$) had a 40% chance of binding.

Discussion

No significant weight gain was found throughout the study, for amitriptyline or trimipramine, at a dosage of 1 mg kg^{-1}, despite many contradictory publications involving tricyclic antidepressants (Remick et al 1982; Berken et al 1984; Fernstrom & Kupfer 1988; Garland et al 1988; Ansseau et al 1989), particularly amitriptyline (Berken et al 1984; Fernstrom & Kupfer 1988). Ansseau et al (1989) reported significant weight gain after only 5 weeks in patients receiving amitriptyline. Garland et al (1988) also reported an average weight gain of 0.57–1.37 kg per month in patients treated with amitriptyline (100–200 mg daily). A study undertaken by Berken et al (1984) showed that a mean increase in weight of 1.3–2.9 lb per month was the net result of six months amitriptyline therapy (maximum doses of 150 mg daily), implying that a greater risk of weight gain is achieved with prolonged tricyclic antidepressant treatment. Fernstrom & Kupfer (1988) also reported amitriptyline-related weight gain after 1 month of therapy in 73 hospitalized patients receiving a dose of 150–300 mg daily. Remick et al (1982) reported significant increase in weight in patients receiving amitriptyline treatment over a 4-week period.

On the other side of the coin, Nakra et al (1977) found no weight gain in 6 healthy subjects after 1 month of treatment with amitriptyline and other tricyclic antidepressants. In another study, rats receiving 2.5 mg kg^{-1} amitriptyline for 20 days displayed no change in caloric intake or any significant weight gain in comparison with a control group (Storlien et al 1985). Nobrega & Coscina (1987) confirm these results in their study, which also subjected rats to amitriptyline treatment in doses ranging from 2.5 to 17 mg kg^{-1}. In Nobrega & Coscina's study, the treatment produced either no significant increase in weight or caloric intake, or slightly reduced levels when compared with a control group.

There are three clear differences between the groups where the investigators reported weight gain or no weight gain: experiments done with depressed patients or healthy subjects/rats; duration of treatment; and dosage of antidepressants given.

Experimentation that resulted in weight gain after amitriptyline therapy focused only on clinically depressed subjects, whereas those studies that did not find weight gain following tricyclic antidepressant treatment used healthy subjects or rats. Ansseau et al (1989), Garland et al (1988), Berken et al (1984), Fernstrom & Kupfer (1988) and Remick et al (1982) all used patients diagnosed with depression as subjects for their studies.

The duration of tricyclic antidepressant therapy may also play a role in whether these drugs are responsible for excessive weight gain. Although Berken et al (1984) reported weight gain after 6 months of antidepressant treatment and Ansseau et al (1989) after 5 weeks, Fernstrom & Kupfer (1988) and Remick et al (1982) reported a significant weight gain in patients after only one month and four weeks, respectively. The healthy subjects used by Nakra et al (1977) in their study did not show any weight gain after 1 month. The rats used in Nobrega & Coscina's (1987) study also did not display any significant weight gain after a month of amitriptyline treatment. In our study the rats did not show any weight gain after 14 weeks and therefore the lack of weight gain was not because of the duration of the experiment.

That no weight gain was found in this study may be due to the relatively low dose of medication administered to the rats, namely 1 mg kg^{-1}. Weight gain was reported by Garland et al (1988), Berken et al (1984) and Fernstrom & Kupfer (1988) when depressed patients used 100–200 mg, 150 mg and 150–300 mg daily amitriptyline, respectively. If the average patient weighs 70 kg, then all these patients received more than 1 mg kg^{-1} per day. However, rats used by Storlien et al (1985) and Nobrega & Coscina (1987), receiving 2.5 mg kg^{-1} daily for 20 days and 2.5–17 mg kg^{-1} daily amitriptyline, respectively, did not gain any weight. In our study, a dose of 1 mg kg^{-1} daily was chosen as this is the dosage given to patients nowadays when tricyclic antidepressants are used to treat depression, pain or sleeplessness. This dosage is lower than any dosage reported to cause weight gain. On the other hand, even a much higher dose of amitriptyline (2.5–17 mg kg^{-1}) given to normal rats by Nobrega & Coscina (1987) did not cause weight gain. It seems, therefore, that 1 mg kg^{-1} of amitriptyline or trimipramine daily does not contribute to any weight gain in normal rats. The dosage of the two tricyclic antidepressants mentioned is, however, responsible for certain metabolic changes, which are highlighted in the following discussion.

Blood glucose levels were found to be significantly increased for both amitriptyline- and trimipramine-treated groups, relative to the control group, for both the 6- and 14-week sacrifice periods despite the rats being starved 12 h before the relative readings being taken. The blood glucose values were not dangerously high but seemed to be on the increase from the 6-week to the 14-week sacrifice period, particularly the trimipramine group. The increased serum glucose levels may be attributed to a significant reduction in muscle and liver glycogen levels, which may be related to the effect that tricyclic antidepressants have on the concentration of noradrenaline in the synaptic cleft. These drugs increase biogenic amine release and block their neuronal reuptake. The net result of this is elevated blood glucose levels brought about by increased glucagon secretion, a direct effect of the biogenic amines. This is, however, speculatory and further investigation is necessary to confirm this. These results contradict the recent findings of Himmerich et al (2006), which describe an increase in glucose uptake associated with decreased serum insulin and cortisol levels in 11 clinically depressed patients receiving mirtazapine. The increased glucose tolerance described may be a result of decreased cortisol levels as well as an increase in physical activity associated with an increase of general well-being brought about by the antidepressant therapy. It has been reported that cortisol levels are slightly increased in untreated depressed patients (Holsboer 2000). Kazes et al (1994) also reported that depression is associated with a decrease in appetite and food intake as well as a significant decrease in physical activity. Antidepressants may be able to improve glucose tolerance and serum insulin levels in clinically depressed patients through normalizing their altered metabolism. Our study,

however, displays the effects antidepressants have on the normal metabolic state within a healthy subject.

Amitriptyline appears to directly promote insulin secretion from INS-1 cells (Figure 3). In the rat study, the results for the circulating serum insulin, however, show the amitriptyline group to have slightly suppressed (but not significant) insulin levels, after 14 weeks of medicational compliance (91.1 ± 11.4 pmol L^{-1}) in comparison with the control group (102.2 ± 14.9 pmol L^{-1}). The tricyclic antidepressant itself may cause an accelerated rate of insulin clearance resulting from the excess insulin being produced and secreted by the β-cells of the pancreas. This possibility is supported by the increased concentration of insulin degrading enzyme found in the liver and muscle of amitriptyline- and trimipramine-treated groups compared with the control group. Computational overlay studies suggest that there is no probability of amitriptyline interaction with the SUR-1 receptor of pancreatic β-cells, whereas trimipramine showed a 40% chance of binding. These results were confirmed when neither amitriptyline nor trimipramine was able to displace [^{3}H]glibenclamide from the SUR-1 receptor of INS-1 cells. These results do not necessarily rule out the possibility that either of these drugs is able to promote insulin secretion indirectly. Amitriptyline and trimipramine may be increasing the ATP:ADP ratio, allowing for more substrate availability for SUR-1 binding. The significant decrease in tissue glycogen found for both test groups may suggest an increase in glycolytic flux providing an increase in ATP levels. Further studies are necessary to confirm this.

An increase in noradrenaline also promotes glycogenolysis, which may be another explanation for the significantly lower tissue glycogen content at both the 6- and 14-week sacrifice period for both test groups in comparison with the controls. The degree of apparent glycogenolysis seems consistent with the blood glucose levels for both time periods, indicating that the excess circulating blood glucose for both test groups is a result of glycogen breakdown. The trimipramine group, which displayed the most significant hyperglycaemia at the 14-week sacrifice, was also found to have the lowest liver glycogen content. A possible reason for lower liver glycogen content, relative to that of the muscle, is a result of the overnight fast.

No difference was found in the glucose clearance experiment for either test group in comparison with the control group. There was, however, slight differences in the distribution of the ^{3}H-labelled deoxyglucose between the control and test groups, though these differences were not significant.

Neither amitriptyline- nor trimipramine-treated rats displayed any difference in appetite compared with control rats. Increased serum glucose levels, among other mechanisms, activate the hypothalamus to decrease appetite. A study was undertaken by Makino et al (2000) to determine the effect of desipramine on the regulation of neuropeptide Y mRNA expression. Desipramine is a tricyclic antidepressant with a similar mechanism of action to that of amitriptyline and trimipramine. Neuropeptide Y is a key neuropeptide that modulates the hypothalamic pituitary adrenal cortex and controls food intake. Neuropeptide Y increases the secretion of corticotrophin-releasing hormone, adrenocorticotropic hormone and corticosterone and also increases food intake (Dallman et al 1993; Stanley et al 1985). Desipramine-treated rats showed a reduction in neuropeptide Y mRNA expression in the arcuate nucleus and a significant decrease of this neuropeptide in the locus coeruleus (Makino et al 2000), thus again implying the reduction in appetite brought about by tricyclic antidepressants.

This study did not measure any neuropeptides and no comments can be made on this aspect, though if the appetite of the rats in this study was not suppressed, the reason may be linked to the dosage of the tricyclic antidepressants the rats received.

INS-1 cell studies and rat serum insulin levels, coupled with insulin degradation studies, suggest that both amitriptyline and trimipramine promote insulin secretion. The accelerated rate of insulin degradation and increased insulin degrading enzyme concentration within the rat imply that the body has increased its rate of insulin clearance to avoid hyperinsulinaemia-associated insulin resistance. The fact that there is no difference in glucose clearance between test and control groups indicates that no insulin resistance is present. Continued overproduction and over-secretion of insulin by the β-cells could result in their apoptosis and subsequent loss of insulin production, which could potentially lead to diabetes.

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