Case Report

Furosemide in the treatment of generalized anxiety disorder: Case report and review of the literature

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Generalised anxiety disorder (GAD) is the most common of the anxiety disorders seen in primary care and the 12-month prevalence in Nigeria may exceed 2.8%. The aim of this case-report is to highlight the use of furosemide in generalised anxiety disorder comorbid with dizziness in an adult female patient. Low-dose furosemide, 20mg to 40mg daily, attenuated the symptoms of generalised anxiety disorder, diagnosed in a young female patient with the Penn State Worry Questionnaire. It ameliorated the symptoms of pessimistic worry, muscle tension, dizziness, easy fatiguability, poor concentration, insomnia and irritability when used alone. Furosemide's action in GAD may be due to its down-regulation of protein kinase C signalling which may be critical in establishing and maintaining a hyperglutamatergic state in key brain areas. Furosemide's action may thus significantly reduce the simplified excitotoxicity index of glutamate/gamma-amino butyric acid. Upregulation of dopamine signalling by furosemide may facilitate contingency awareness and the assigning of motivational value or valence to unexpected events that may also be critical for the prevention of generalised anxiety. The safety profile of furosemide and low cost warrants its being further explored in GAD, a disease that still exerts much toll on human health since present medication is only effective in a fraction of patients.

Keywords: Generalised anxiety disorder, furosemide, glutamatergic signalling, protein kinase C, neural circuitry.

INTRODUCTION

Anxiety disorders are the most prevalent of all psychiatric disorders with generalised anxiety disorder (GAD) being the most common of the anxiety disorders to be seen in primary care (Davidson et al., 2010). The 12-month prevalence rate of GAD has been reported to be low (0%) in Nigeria (Gureje et al., 2006) and higher (8%) in America (Davidson et al., 2010) though a 2.8% prevalence rate has been found in another Nigerian series (http://www.unthenugu.org, 2010). GAD occurs more in women, the sex ratio being 2 women to 1 man (Brawman-Mintzen and Lydiard, 1996) and approximately fifty percent of cases begin in childhood or adolescence

(http://www.surgeon-general.gov./library/mentalhealth).

GAD, an anxiety disorder without an external trigger, may be defined by a chronic (> 6 months duration) period of free-floating anxiety (Kalk et al., 2011) and pessimistic worry, accompanied by multiple associated symptoms. These symptoms include muscle tension, easy fatiguability, poor concentration, palpitations, insomnia and irritability. In GAD, there is heightened intensity of emotional experience;   poor understanding of emotions, for example, relative inability to identify discrete emotions and to access and utilise the adaptive information conveyed by one's emotions as a source of knowledge. There is also a negative reactivity to one's emotional state, for example, fear of emotion and maladaptive emotional management responses that engages worry, which is a central feature of GAD (Turk et al., 2005). The excessive worries often pertain to many areas, including

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work relationships, finances, the well-being of one's family, potential misfortunes and impending deadlines. Fear of internal experiences and fear of losing control over one's emotions may be associated with the severity of GAD symptoms.

The aetiology of anxiety as noted by Barlow (2000) implicates a tripod of vulnerabilities which are, firstly, generalised biological or heritable vulnerability; secondly, generalised psychological vulnerability based on early experiences in developing a sense of control over salient events, and thirdly, more specific psychological vulnerability in which one learns to focus anxiety on specific objects or situations.

Genetics and GAD

GAD and major depression are influenced by the same genetic factors (Kendler et al., 1992). Genetic association studies have identified polymorphisms in genes such as glutamate receptor, ionotropic, N-methyl-D-aspartate ($GRIN_1$, $GRIN_2B$), corticotrophin-releasing hormone (CRH), glutamate transporter (SLC_1A_1), adenosine A_{2A} receptor ($ADORA_{2A}$), regulator of G-protein signalling (RGS_2), early growth response (ERG_2), serum/glucocorticoid regulated kinase (SGK_1), cholecystokinin receptor (CCR_5), dopamine receptor (DRD_1), nuclear receptor, subfamily 4, group A, member 2 (NR_4A_2), gamma-amino butyric acid-B receptor (GABA(B)R, prostaglandin D_2 synthase (PTGDS), quaking homolog, KH domain, RNA binding protein (QK_1), delta-aminolevulinate dehydratase (ALAD), dynein light chain$_2$ ($DYNLL_2$) and GABA-B_R which may be important in the pharmacogenomics of anxiety (Le-Niculescu et al., 2011; Donner et al., 2008; Stoppel et al., 2006). Candidate biomarkers for anxiety include $DYNLL_2$, SGK_1 and CCR_5.

Neural circuitry in anxiety

The amygdala

The amygdala through the basolateral nucleus (BLA) integrates inputs from the various brain regions to produce a coherent behavioural response to fear and anxiety. Adolescents with generalised anxiety disorder (GAD) manifest alterations in amygdala circuits involved in emotion processing (Roy et al., 2012). The function of BLA is regulated by the prefrontal cortex (PFC), thalamus and hippocampus and BLA projects to the orbitofrontal cortex, hippocampus, dorsal and ventral striatum, central nucleus of amygdala and lateral extended amygdala which includes central and lateral amygdaloid nuclei, bed nucleus of the stria terminalis (BNST) and sublenticular substantia innominata (Davis and Whalen, 2001). The central nucleus of the amygdala (CeA) is an output

nucleus of the amygdala and its lesions prevent anxiety responses (Davis, 2002). The CeA comprises the interface between the amygdala and the motor, autonomic and neuroendocrine systems involved in the expression of anxiety behaviour.

The bed nucleus of the stria terminalis

The hypothalamic and brain stem structures that mediate the expression of emotional behaviour can also be activated directly by the BNST. The BNST is an important component of anxiety circuitry and examples of its target areas include the parabrachial nucleus, the lateral hypothalamus, ventral tegmental area, locus coeruleus and nucleus ambiguus. It may also function as a relay between limbic cognition centres and reward, stress and anxiety nuclei (McElligott and Winder, 2009). BNST has rich variety of neuropeptides and neuropeptide receptors, for example, calcitonin gene-related peptide (CGRP) and pituitary adenylate cyclase activating peptide (PACAP) mediating anxiety-like patterns of behaviour (Sink et al., 2013; Hammack et al., 2010) and there is a rich innervation from the parabrachial nucleus and paraventricular nucleus of the hypothalamus (PVN) to the BNST expressing CGRP and PACAP (Kainu et al., 1993; Hammack et al., 2010). The anxiogenic effects of both CGRP and PACAP may be mediated by corticotrophin-releasing hormone (CRH); while activation of alpha$_2$-adrenergic receptors in the BNST decreases glutamatergic transmission (Shields et al., 2009).

Higher cortical regulation of amygdaloid activity

The PFC structures are thought to participate in interpreting the higher-order significance of experiential stimulus, in modifying behavioural responses based on competing reward versus punishment contingencies and in predicting social outcomes of behavioural responses to emotional events (Davis, 2002). Areas of the PFC involved in emotional processing are the pregenual anterior cingulate cortex, the orbital and anterior insular cortex and the posterior cingulate cortex. For example, the pregenual anterior cingulate-amygdala circuitry is important in emotional reactivity and is disrupted in GAD (Nauert, 2010).

The autonomic system in anxiety

Stimulation of the lateral nucleus of hypothalamus by afferent projections from the CeA, the BNST or ventral striatum activates the sympathetic nervous system. Stress stimulates CRH release from paraventricular nucleus of the hypothalamus and amygdala. Parasympathetic vagus and splanchnic nerves receive

afferent projections from lateral hypothalamus, the paraventricular nucleus, the locus coeruleus, the amygdala, the infralimbic prefrontal cortex and the prelimbic posterior anterior cingulate cortex.

Glutamatergic signalling in anxiety

Glutamate acts on three different cell compartments (presynaptic neurons, postsynaptic neurons and glia) that characterise the 'tripartite glutamatergic synapse' and the glutamate-glutamine cycle plays a key role in the regulation of synaptic plasticity (Zarate et al., 2010). Down-regulation of the excitatory amino acid transporter (EAAT) and the vesicular glutamate transporter (VGLUT) is associated with anxiety.

A hyperglutamatergic state may be implicated in GAD due to the imbalance of excitation/inhibitory input to the locus coeruleus. There is loss of glutamate-GABA harmony in GAD (Wieronska et al., 2011) due, most probably, to imbalance of the activities of the main inhibitory input to the locus coeruleus, the nucleus prepositus hypoglossi (Ennis and Aston-Jones, 1989), and the major excitatory input, the nucleus paragigantocellularis (PGi) (Ennis and Aston-Jones, 1988). PGi stimulation, which may become dominant in an allostatic state, increases extra-cellular glutamate concentration within the locus coeruleus (Liu et al., 1999) and excitatory amino acids may operate primarily at a kainite-type receptor on locus coeruleus neurons to effect excitation from PGi. Proposed excitatory amino acid (EAA) pathway from PGi may serve as a final link in a variety of secondary input to the locus coeruleus (LC) (Ennis and Aston-Jones, 1988; Aston-Jones et al., 1991). Glutamate via N-methyl-D-aspartate receptors promotes the release of neuropeptide FF (NPFF), an anti-opioid peptide, from rat spinal cord slices (Devilliers et al., 1994).

Protein kinase C signalling in GAD

Protein kinase C (PKC), the calcium- and phospholipid-dependent enzyme system, which plays a pivotal role in cell signalling systems (Hahn and Friedman, 1999), is able to phosphorylate N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor sites and mGluR5 receptor sites (Szabo et al., 2009; Lee et al., 2008), It is anxiogenic (Bowers et al., 2000) and regulates many aspects of mGluR function including Ca^{2+} signalling. PKC inhibitors are able to suppress glutamate uptake by glutamate/aspartate transporter (GLAST) (Bull and Barnett, 2002). Free intracellular calcium (Hassam et al., 1997) and neurotoxic concentrations of glutamate (Hassam et al., 1997) activate PKC which may be a mediator of glutamate-induced neurotoxicity. Thus PKC,

which also has a depressogenic effect in the forced swim test, may be a critical factor in establishing and maintaining a hyperglutamatergic state. The anxiogenic peptides cholecystokinin (CCK) (Deng et al., 2010), calcitonin gene-related peptide (CGRP) (Kainu et al., 1993; Sink et al., 2011, 2013), pituitary adenylate cyclase-activating peptide (PACAP) (Hammack et al., 2009, 2010); substance P (Werry et al., 2006), orexin (Uramura et al., 2001) and corticotropin-releasing hormone (CRH) (Refojo et al., 2011; Hollrigel et al., 1998) induce glutamate activation and may significantly increase the simplified excitotoxicity index of glutamate/GABA.

The cannabinoid system, PKC and GAD

Though cannabinoid CB1 and, especially, CB2 receptor activation may attenuate excitotoxic glutamatergic neurotransmission and are reported to be neuroprotective (Palazuelos et al., 2009), upregulation of cannabinoid CB2 receptors has been found associated with anxiety and its chronic blockade found to be anxiolytic (Garcia-Gutierrez et al., 2012). This may be responsible for the reported link between cannabiods and anxiety (Bunn, 2012; Franklin and Carrasco, 2013) which finds explanation in the report by Partland (2001) and Bouaboula et al (1996) that cannabinoid CB2 agonists may signal through PKC and upregulate 5-HT(2A) receptor signalling via phospholipase C beta (PLCβ) activity in prefrontal cortex (Franklin and Carrasco, 2013).

The cation-chloride-cotransporters and PKC

There is stimulation of the sodium-potassium cotransporter (Na^+-K^+-2Cl) activity by group 1 and group 2 mGluRs which is antagonised by furosemide (Sun and Murali, 1998). Also, $mGluR_{1-}$ and $mGluR_5$- (group 1 mGluRs) induced PKC phosphorylation of the potassium-chloride cotransporter$_2$ (KCC_2) is blocked by furosemide (Banke and Gegelashvili, 2008) which also reduces metabotropic glutamate-induced astroglial cell swelling (Hansson, 1994). Thus, furosemide, which selectively inhibits GABA-gated currents in α6-expressing cells (Hoffpauir and Gleason, 2002) may function overall to decrease the excitotoxicity index of glutamate/GABA.

Noradrenergic signalling in GAD

There is increased noradrenergic transmission from the noradrenergic LC leading to increased excitation of brain areas involved in GAD. Though, there is little clinical evidence supporting a primary role for the noradrenergic system in the pathophysiology of GAD and the anti-adrenergic agent, propranolol may have no place in treat-

ment of GAD (Paris et al., 2006). Plasma methylhydroxy phenylglycol (MHPG) levels, resting plasma norepinephrine in GAD have not been consistently reported to be elevated (Charney et al., 1995).

Functional interactions among different neurotransmitters (Vizi et al., 2004) and neuropeptide systems and neuroanatomic and functional inter-relations amongst brain structures such as the amygdala, hypothalamus, hippocampus and LC may be more important in responsible for different components of anxiety disorder syndromes. For example, interactions between the dorsal raphe nucleus and LC is a potential substrate for co-regulation of noradrenergic, serotonergic and non-serotonergic influences on the sensorimotor, interoceptive and cognitive components of co-morbid balance, migraine and anxiety (Balaban et al., 2011). Furthermore, noradrenaline, cortisol and CRH appear tightly linked as a functional system that offers homeostatic mechanisms for responding to stress (Charney and Drevets, 2002). Coordinated functional interactions between the hypothalamo-pituitary-adrenal (HPA) axis and the noradrenergic systems play major roles in producing adaptive responses to stress. Noradrenaline release increases CRH release from the paraventricular nucleus of the hypothalamus and CRH release increases LC neuronal firing activity.

Serotonergic signalling in GAD

Serotonergic nuclei are found in the rostral and caudal raphe nuclei. Neurons expand from the rostral raphe to the cerebral cortex, limbic regions and basal ganglia where serotonergic transmission is decreased in GAD. Hypofunction of the serotonergic neurons arising from the dorsal raphe nucleus lead to lack of inhibitory effect on the GAD pathway resulting in hyperactive neurotransmission circuits between the cortex, thalamus, amygdala and hypothalamus (Roy et al., 2012). The overactivity of stimulatory pathways is hypothesized to generate GAD (Coplan et al., 1995). Activity of descending neurons to brain stem is unaffected in GAD.

GABAergic signalling in GAD

Activity of GABAergic neurons is decreased in GAD. This decrease in GABAergic neurotransmission is seen at all areas of the CNS including the hypothalamus, hippocampus, cerebral cortex and cerebellar cortex. This may lead to lack of inhibitory effect on the GAD pathway. This may be one of the reasons for the loss of glutamate-GABA harmony in anxiety with unopposed action of excitatory pathways (Wieronska et al., 2011), for example, from the PGi to the LC. Drugs such as furosemide which may induce serotonin release in emotion pathways (De Gobbi et al., 2007) could depola-

rise GABAergic terminals via 5-HT (2A) receptor-mediated action, thereby decreasing the simplified excitotoxicity index of glutamate/GABA.

Dopaminergic signalling in GAD

Activation of dopamine neurons by restoration of functional NMDAR to dopamine neurons in ventral tegmental area (VTA) is critical for the prevention of the development of GAD (Zweifel et al., 2011). Dopamine has a role in facilitating contingency awareness that is critical for the prevention of generalised anxiety. Impairment in dopamine-dependent salience or attention-orienting signal detection prevents development of the appropriate conditioned stimulus-unconditioned stimulus association which results in failure to associate a predictive stimulus with the aversive outcome. Dopamine neurons of the ventral midbrain help to facilitate adaptation to environmental changes and assigning of motivational value or valence to unexpected events (Zweifel et al., 2011).

Interaction between neuropeptide Y and corticotropin-releasing hormone in GAD

There are opposing roles for CRH and neuropeptide Y (NPY) in the regulation of stress/anxiety-related behaviours. Re-establishing homeostasis in response to a challenge or allostatic balance may depend on the opposing roles for NPY and CRH within the amygdala (Thorsell, 2010).

Brain-derived neurotrophic factor in GAD

The neurotrophic protein, brain-derived neurotrophic factor (BDNF), which expression is low in depression is also low in GAD, obsessive-compulsive disorder and social anxiety disorder (Wang et al., 2009). BDNF increases dynorphin levels (Logrip et al., 2008) which may have relevance in anxiety for mice with disabled dynorphin gene exhibit persistent anxiety (Bilkei-Gorzo et al., 2012). BDNF directly stimulates expression of dopamine D3 receptors mRNA and also induces the expression of the anxiolytic peptides, NPY and enkephalin (Bamea et al., 1995; Bamea and Roberts, 2002; Saylor and McGinty, 2008). There is a positive feed-back loop between the endocannabinoids and BDNF for BDNF regulates neuronal sensitivity to endocannabinoids (eCBs) and eCBs can in turn regulate BDNF expression and function (Maison et al., 2009).

Current drug treatment of GAD

The treatment goals in GAD are to reduce the core symp-

toms, both the psychic and the somatic; to improve patient function and quality of life; to treat co-morbid disorders present at the time of diagnosis and those that appear over the long term; to continue treatment for long enough to produce remission and prevent relapse.

Present drug treatment for GAD, which includes the following, is effective in only about 39% of patients and there is low likelihood of complete remission (Davidson et al., 2010)

i. The selective serotonin reuptake inhibitors (SSRIs) such as escitalopram, sertraline and paroxetine; and the serotonin-noradrenaline reuptake inhibitor (SNRI) venlafaxine are presently first-line drugs for the treatment of GAD (Paris et al., 2006; Baldwin et al., 2005; Kavan et al., 2009). They are able to suppress the symptoms of anxiety and the comorbid depression present in up to 30-45% of patients with GAD. The SSRIs and 5-hydroxy-trptophan transiently worsen GAD due to increased synaptic cleft 5-HT until adaptive receptor changes have taken place (Coplan et al., 1995) and this can be prevented by co-administration of the benzodiazepines. Delay in onset of action is their major draw-back.

ii. The benzodiazepines such as lorazepam may also be first-line drugs for the treatment of GAD. They minimise the somatic symptoms of GAD but have less effect on the key psychological aspects (Bandelow et al., 2008).

iii. The azapirones,n such as buspirone, are partial agonists of the 5-HT1A receptor. They are as effective as the benzodiazepines in GAD and also effective in depression; have no withdrawal symptoms and with low potential for abuse unlike the benzodiazepines (Bandelow et al., 2008).

iv. Other drugs for treatment of GAD include the tricyclic antidepressant, imipramine, which is a second line drug; the histamine H1 receptor antagonist, hydroxyzine, which is a second line drug and the alpha2 δ calcium channel modulator, pregabalin, which increases glutamic acid decarboxylase activity and decreases glutamate release (Strawn and Geracioti, 2007). NMDAR blockers such as intravenous ketamine (Zarate et al., 2010), AMPAR antagonists, mGluR1,2 antagonists, mGluR5 negative allosteric modulators such as fenobam, mGluR3 agonists such as eglumegad and mGluR4 agonists and positive allosteric modulators of mGluR7 display anxiolytic effects and are prospective drugs (Wieronska et al., 2011). Riluzole inhibits glutamate release, enhances glutamate uptake by EAATs and GLAST and increases AMPA trafficking. Atypical antipsychotics such as olanzapine may be used in combination with an SSRI like fluoxetine in resistant cases of GAD. Selective β-blockade such as with propranolol has little to play in the treatment of anxiety and is not recommended (Paris, 2006; Meibach et al., 1987; Tyrer et al., 1991).

Diagnosis of GAD

The Hamilton Anxiety Rating Scale has limitations in the diagnosis of GAD as it does not adequately assess the excessive and uncontrollable worry characteristic of GAD (Koerner et al., 2010). The Penn State Worry Questionnaire is a 16-item inventory designed to diagnose GAD. It captures the generality, excessiveness and uncontrollability characteristic of pathological worry which is the central feature in GAD. It has good internal consistency and test-retest reliability (Turk et al., 2005).

PSWQ has 16 items and each item is rated on a scale from 1 ('not at all typical of me') to 5 ('very typical of me'). Eleven items are worded in the direction of pathological worry, with higher numbers indicating more worry (e.g., 'Once I start worrying, I cannot stop'), while the remaining five items are worded to indicate that worry is not a problem, with higher numbers indicating less worry (e.g., 'I never worry about anything'). Total score is calculated by summing the first 11 items and the reverse-scores of the latter 5 items, with higher PSWQ scores reflecting greater levels of pathological worry. (Table 1)

Scoring the PSWQ

In scoring the PSWQ, a value of 1, 2, 3, 4 and 5 is assigned to a response depending upon whether the item is worded positively or negatively. The total score of the scale ranges from 16 to 80 with a cut-off score of 62 for GAD (Meyer et al., 1990).

Item 1,3,8,10,11 are reverse scored as follows:
- Very typical of me = 1 (circled 5 on the sheet)
- Circled 4 on the sheet = 2
- Circled 3 on the sheet = 3
- Circled 2 on the sheet = 4
- Not at all typical of me = 5 (circled 1 on the sheet)

For item 2,4,5,6,7,9,12,13,14,15,16, the scoring is:
Not at all typical of me =1.Ratings of 2, 3, and 4 are not transformed.Very typical of me = 5

CASE PRESENTATION

Mrs. R. A. is a 36 year old house wife, who lives in Ekpoma, Edo-State, Nigeria. She presented to us towards end of April, 2012 with a history of 'hypertension', dizziness and uncontrollable worry of 12 months duration. The worry was about her health, husband's fidelity, finances and children's poor grades in school. She also complained of insomnia, difficulty in concentration, easy fatiguabilitry, restlessness and muscle tension. She believed she had hypertension and appendicitis. She was euthyroid and had no symptoms of phaeochromocytoma. Generalized anxiety disorder was confirmed using the American Psychiatric Association's Diagnostic and Statistical Manual IV Text

Table 1. The Penn State Worry Questionnaire (PSWQ).

		Not at all typical of me			Very typical of me	
1.	If I do not have enough time to do everything, I do not worry about it.	1	2	3	4	5
2.	My worries overwhelm me.	1	2	3	4	5
3.	I do not tend to worry about things.	1	2	3	4	5
4.	Many situation make me worry.	1	2	3	4	5
5.	I know I should not worry about things, but I just cannot help it.	1	2	3	4	5
6.	When I am under pressure I worry a lot	1	2	3	4	5
7.	I am always worrying about something.	1	2	3	4	5
8.	I find it easy to dismiss worrisome thoughts.	1	2	3	4	5
9.	As soon as I finish one task, I start to worry about everything else I have to do.	1	2	3	4	5
10.	I never worry about anything.	1	2	3	4	5
11.	When there is nothing more I can do about a concern, I do not worry about it anymore.	1	2	3	4	5
12.	I have been a worrier all my life.	1	2	3	4	5
13.	I notice that I have been worrying about things.	1	2	3	4	5
14.	Once I started worrying, I cannot stop.	1	2	3	4	5
15.	I worry all the time.	1	2	3	4	5
16.	I worry about projects until they are all done.	1	2	3	4	5

Revision (DSM-IV-TR) and Penn State Worry Questionnaire (PSWQ) (Meyer et al., 1990; Fresco et al., 2003) which met the following criteria
1. Excessive worry (apprehensive worry)
2. Patient found it difficult to control the worry
3. The anxiety and worry were associated with:
a) sleep disturbance
b) muscle tension
c) restlessness
d) feeling of easy fatigability.

With the PSWQ, patient scored 67 confirming GAD as the cause of the anxiety (Turk et al., 2005), after exclusion of co-morbid associations (see below). Since there are no specific tests to diagnose GAD, the following physical examination and laboratory tests were done to rule out possible comorbid medical illnesses:

She was normotensive with BP of 120/75 mm Hg. Pulse was 72/min with respiratory rate at 25/min. Temperature was normal at 36.8˚C. Systemic and neurological examinations, including ophthalmic and otological examinations, were normal. The investigations done included:
1. Complete blood count, which was normal
2. Urinalysis, which was normal
3. Serum electrolytes and urea, which were normal
4. Malaria parasite test, which was negative
5. Widal test, which was negative
6. Fasting blood sugar, which was normal at 78mg/dl
7. A routine chest X-ray, which revealed no abnormality
8. Skull X-ray which was normal
9. Ultra-sound examination of cranial vessels was normal
10. Haemoglobin genotype, which was rhesus positive

In May 2012 she was placed on short term furosemide (40mg/day), NAFDAC number 04 – 9146) because she could not tolerate hydrochlorothiazie. She reported eight days later that her excessive   worry, dizziness, inability to concentrate, sleep disturbances, easy fatiguability and restlessness had improved since she was started on furosemide, and the PSWQ became 30 and remained so for the next 12 weeks after which the dose of furosemide was reduced to 20mg every other day. Patient has remained stable with no symptom of dizziness, insomnia and with disappearance of the worry.

Exclusion of comorbid medical illnesses

Patient did not abuse alcohol or any other substance likely to cause addiction. Patient was well-oriented in time and space and had no tremors or dyskinesia. There was no poverty of movement or rigidity.

The Penn State Worry Questionnaire (PSWQ) has proven a valid psychometric test to diagnose anxiety disorders such as GAD. The score of 67 which this patient had is diagnostic for GAD (Turk et al., 2005; Zhong et al., 2009) and excluded social anxiety and PTSD. Pessimistic worry is more a feature of GAD (generalised anxiety disorder) than for PTSD or social phobia.

The Geriatric Depression Scale (GSD) (Mancusco et al., 2000), administered as part of a structured face-to-face interview, was used to rate the depression after its diagnosis by the diagnostic and statistical manual (DSM) of the American Psychiatric Association. Here, the patient scored 11 just above the cut-off of 10 and this suggested mild depression.

Furthermore, the General Practitioner Assessment of Cognition (GPCOG) (Brodaty et al., 2004), a revised version of the Mini-Mental State Examination, in which the patient scored 9 ruled out cognitive impairment.

DISCUSSION

Case report supports the use of furosemide in anxiety disorders which we found serendipitously in 2007 (Personal Communication). In this present report, furosemide attenuated the symptoms of GAD significantly at 12 weeks. The beneficial effect of furosemide in anxiety in rats reported by Krystal et al (2012) and the anti-depressant effect of furosemide in mice reported by Oriaifo and Omogbai (2011) lend support to this case report, for anxiety and depression frequently co-exist.

CRH is upregulated in GAD (Thorsell, 2010) and contributes to the glutamate-GABA disharmony in this condition. The upregulation of CRH may be due to PKC (Lesscher et al., 2010) and the synthetic PKC antagonist, furosemide, may block this PKC-induced rise in CRH. The activation of Bax (Chan et al., 2007) and the translocation of KCC2 to the cell membrane by PKC (Lee et al., 2012) are blocked by furosemide.

Also, furosemide (Lundy et al., 2003) and CRH (De Castro e Silva et al., 2006) have opposite effects on salt-appetite. The induction of salt-appetite by furosemide may enhance the release of enkephalin which may be antidepressant/anxiolytic (Grondin et al., 2011; Zozulya et al., 2009; Comings et al., 2000) and enhance the synthesis of BDNF. Also, PKC-induced release of CRH will be decreased in the process. Apart from inhibiting the induction of salt-appetite, CRH may enhance glutamatergic neurotransmission which enhances the release of the anti-opioid peptide, neuropeptide FF (Devilliers et al., 1994), an activity furosemide may oppose.

Furosemide may attenuate PKC-induced enhancement of glutamatergic neurotransmission (Hassam et al., 1997; Szabo et al., 2009; Bull and Barnett, 2002) and induction of PKC by the anxiogenic peptides CRH, CGRP, PACAP, substance P and cholecystokinin. It may also down-regulate glutamatergic neurotransmission by the nucleus paragigantocellularis since it blocks calcium influx through kainate, AMPA and NMDA receptors (Sanchez-Gomez et al., 2011; Beck et al., 2003). Furosemide may antagonise glutamate-induced astroglial cell swelling (Hansson et al., 1994), reduce ATP-induced release of glutamate (Jeremic et al.,

2001) and reduce the trimethytin-evoked glutamate efflux (Patterson et al., 1996).

Furosemide antagonises the anxiogenic peptides and decreases their mediation of glutamate release. Various workers have reported that furosemide antagonises CGRP (McCulloch and Cooke, 1989); PACAP (Kuwahara et al., 1993); cholecystokinin (Xiao et al., 2012), substance P (Matowe et al., 2007) and adenosine (Gottlieb et al., 2000).

BDNF is anxiolytic (Wang et al., 2009) and furosemide induces its synthesis (Szekeres et al., 2010). Furosemide, which has a BDNF-mimetic action in down-regulating KCC2, may synergise with BDNF in the enhancement of the release of the anxiolytic peptides, neuropeptide Y (NPY) (Croll et al., 1979), enkephalin and dynorphin. There is a direct interaction between the anxiolytic NPY and the anxiogenic CRH systems within the BLA to modify anxiety-related behaviour through differential regulation of cAMP levels (Thorsell, 2010) with NPY increasing cAMP levels and CRH producing the opposite effect (Sheriff et al., 2001). Also, by down-regulating the norepinephrine transporter (Habecker et al., 2003) and dopamine transporter (Lucas et al., 2003), furosemide indirectly increases dopamine and BDNF levels. Importantly, dopamine in the mesolimbic dopamine system is important for aversive conditioning and the prevention of generalised anxiety, the disruption of which results in sensitisation of the acoustic startle response (ASR) (Zweifel et al., 2011).

Serotonergic 5-HT(1A) receptor agonists are anxiolytic (Krystal and Neumeister, 2009; Coplan et al., 1995; Gross et al., 2002) and furosemide can activate this subtype of receptors in the lateral parabrachial nucleus (LPBN) (De Gobbi et al., 2007) and can also induce the release of serotonin from the LPBN which is antagonised by the NMDA antagonist MK-801 showing the involvement of NMDA receptor mechanisms in the modulation of serotonin release (Tanaka et al., 2006). This increase in serotonin release which may be mediated by furosemide could reduce locus coeruleus activity via 5-HT(2A) receptor-mediated depolarisation of GABAergic terminals both in the locus coeruleus-vestibular and LPBN-vestibular pathways to attenuate the symptom of dizziness which this patient had (Balaban et al., 2011; Kainu et al., 1993). Additionally, 5-HT receptor activation by furosemide may cause repression of the anxiogenic calcitonin gene-related peptide promoter (Durham et al., 1997) and also repression of its activation by mitogen activated protein (MAP) kinase (Durham and Russo, 1998).

In conclusion, case report and literature review show that furosemide may be important for GAD, and warrants being further explored.

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