

Differential effects of sertraline, imipramine, nifedipine, furosemide and bumetanide on monoaminergic signalling

*¹Oriaifo S. E., ²Omogbai E. K. I. and ³Nicholas Oriaifo

¹Department of Pharmacology and Therapeutics, College of Medicine, Ambrose Alli University, Ekpoma

²Department of Pharmacology, University of Benin, Benin City

³Department of Pediatrics, Irrua Specialist Teaching Hospital, Irrua

The aim of the study was to identify the pattern of active behaviours exhibited by the agents' sertraline, imipramine, nifedipine, furosemide and bumetanide in the forced swim test (FST) using mice. Six groups of mice (six mice in each group) were used in the study of swimming times and climbing times. A control group received Tween 80. Other groups were treated with imipramine (10mg/kg), sertraline (5mg/kg), nifedipine (5mg/kg), furosemide (10mg/kg) and bumetanide (2.5mg/kg) acutely, subchronically for 14 days and chronically for 30 days. A study with the FST was done acutely, on the 15th day and on the 31st day. Results showed that sertraline prolonged swimming times and in the same breath decreased climbing times acutely, subchronically and chronically significantly ($P < 0.05$, < 0.01) compared to control values. Imipramine prolonged swimming times acutely, subchronically and chronically but increased climbing times only when given chronically. Nifedipine prolonged swimming times significantly only when given subchronically and chronically, and did not affect climbing times. Bumetanide only prolonged swimming times significantly when given chronically while furosemide did not affect swimming times but prolonged climbing times acutely, subchronically and chronically significantly when compared to control values ($P < 0.05$, < 0.01). In conclusion, the results suggest that serotonin may affect multiple and complex signal transduction pathways; imipramine the serotonergic and noradrenergic signalling, nifedipine and bumetanide the serotonergic signalling and furosemide the noradrenergic signalling for their respective effects.

Keywords: Imipramine, sertraline, nifedipine, bumetanide, furosemide, climbing times, swimming times, FST.

INTRODUCTION

Different antidepressant drugs exhibit varying patterns of active behaviours in the forced swim test. Multiple signals

List of abbreviations

SD: Standard deviation; FST: Forced swim test; KCC₂: Isoform 2 of the neuron-specific potassium-chloride symporter; NKCC₁: Isoform 1 of the sodium-potassium-chloride co-transporter; BDNF: Brain-derived neurotrophic factor; GABA: Gamma-amino butyric acid; CCB: Calcium channel blocker; SERT: Serotonin transporter; TCA: Tricyclic antidepressant; SSRI: Selective serotonin reuptake inhibitor; NMDA: N-Methyl-D-aspartate

transduction pathways or signalling systems are implicated in the mechanism of action of antidepressants.

The notable ones are the serotonergic, noradrenergic and the dopaminergic systems. There is co-operativity amongst the various monoaminergic signaling systems (Thomas et al., 1998) which are modulated by the cholinergic, the glutamatergic, and the gamma-aminobutyric acid- (GABA)-ergic systems. The latency of action of antidepressants has been ascribed to the down-stream neuro-adaptive changes.

Lucki (1997) showed that different patterns of active behaviours are elicited in the FST depending on whether the test compound enhanced noradrenergic or dopaminergic signalling (more of climbing or struggling) or serotonergic signalling (more of swimming) and that

these could be scored.

Calcium channel blockers (CCBs) have antidepressant-like properties (Mogilnicka et al., 1987; Czyrak et al., 1990; Tazi et al., 1992). Nifedipine is known to inhibit calcium channel, serotonin reuptake (Wendling et al., 1987), dopamine reuptake (Reiriz et al., 1994) and may also affect noradrenaline reuptake (Terland, 1992).

Furosemide, which has been reported to possess antidepressant-like effects (Oriaifo and Omogbai, 2010), affects GABAergic signalling through its effect in down-regulating a neuron-specific potassium-chloride symporter, KCC₂, and may synergise with brain derived neurotrophic factor (BDNF) (Wardle and Poo, 2003) in this regard. It has significant actions against adenosine (O'Connor et al., 1991) and inhibits phosphodiesterase (Marcus et al., 1978). It can inhibit the dopamine transporter (Lucas et al., 2007) and chronic furosemide may lead to inhibition of the norepinephrine transporter (Habecker et al., 2003). Furosemide stimulates renin release (Hummerich et al., 1981) which enhances the production of angiotensin II. Bumetanide predominantly inhibits the isoform 1 of the sodium-potassium-chloride co-transporter, NKCC1, and also induces angiotensin release (Scholz et al., 1993). Both bumetanide and furosemide prevent sustained Ca²⁺ increases and are anti-apoptotics, actions which may positively affect neuroplasticity. Both chloride transport blockers also prevent N-methyl-D-aspartate- (NMDA)-receptor channel activation and glutamate-mediated excitotoxicity which also explains their antidepressant-like properties.

Imipramine and sertraline have been in use as antidepressants for over fifty and twenty years respectively. They are known to inhibit monoamine transporters (Holmes et al., 2001) and increase the level of BDNF which is low in depression. Imipramine (a tricyclic antidepressant) affects both the norepinephrine and serotonin transporters while sertraline is an antidepressant of the selective serotonin reuptake inhibitor class (SSRI).

The aim of the study was to determine the patterns of activity exhibited, whether swimming or climbing, by furosemide, bumetanide and nifedipine and compare them to that of the known antidepressants, imipramine and sertraline.

MATERIALS AND METHODS

Six groups (six mice each) of male albino mice (25-35g) participated in the study of swimming times. Also, six groups (six mice each) of male albino mice (25-35g) participated in the study of climbing times. The mice were housed in the Animal House for acclimatisation (where they were fed *ad libitum*) for two weeks before transfer to the sound-proof testing-area in their own cages. Mice

were pretreated intraperitoneally with 0.25ml of Tween 80 as control group, sertraline (5mg/kg), imipramine (10mg/kg), nifedipine (5mg/kg), furosemide (10mg/kg) and bumetanide (2.5mg/kg) and acute study was done. They were then pretreated for 14 days and subchronic study was done. Pretreatment was continued for 30 days and chronic study was carried out. The same protocol was undertaken both for the swimming studies and for the climbing studies. On test days, they were allowed to adapt for one hour before administration of the test compounds intraperitoneally and one hour was allowed to elapse before subjecting them to the FST to know the pattern of active behaviour. All the drugs were supplied by Sigma-Aldrich through Rovet Chemicals, Benin-City, Nigeria. The doses of drugs were chosen from previous studies (Eraly et al., 2006; Luszczki et al., 2003; Cryan et al., 2004; Kosuda et al., 1997; Hesdorffer et al., 2001; Mogilnicka et al., 1987) and test compounds were dissolved in 10% Tween 80 because of furosemide's solubility.

Drug Studies with the forced swim test

Mice were forced to swim for five minutes in a vertical glass cylinder of height 46cm, diameter 20cm and containing fresh tap water to a depth of 30cm (Detke and Lucki, 1996) at 27°C. The mice were dried and kept warm after each test session by the author. A behavioural model of immobility first postulated by Porsolt (Porsolt et al., 1977) and named the behavioural despair model was used. In this model, mice are forced to swim in a restricted space from which escape is not possible. Following an initial period of vigorous activity, the mice become helpless and adopt a characteristic immobile posture with no further attempt to engage in escape-related behaviour; and this reflects a state of despair or lowered mood. This is the core behaviour but before the mice get immobile, other components of antidepressant drug responses were observed and timed by an observer unaware of the drug administered. In the five-minute swim session, the swimming time defined as movement throughout the swim chamber was timed. Also, climbing activity (also termed thrashing) which consisted of upward directed movements of the forepaws along the side of the swim chamber was also timed.

Records of swimming times and climbing times were done at (a): beginning of first week (acute); (b): on Day 15 (subchronic) and (c): on Day 31 (chronic)

Statistical Analysis

The Mann-Whitney non-parametric test for comparing two groups was used for statistical analysis and difference was considered significant ($P < 0.05$, < 0.01)

Table 1. Effects of acute, subchronic and chronic sertraline, imipramine, nifedipine, bumetanide and furosemide on swimming times in mice.

Drug	Acute	P	Subchronic	P	Chronic	P
Control	30.36 ± 1.6		30.40 ± 3.5		30.50 ± 3.9	
Sertraline	72.43 ± 1.8	< 0.01	80.18 ± 3.3	< 0.01	100.00 ± 3.7	< 0.01
Imipramine	60.66 ± 4.4	< 0.01	60.32 ± 4.6	< 0.01	50.64 ± 2.4	< 0.01
Nifedipine	30.24 ± 3.8	> 0.05	40.56 ± 3.4	< 0.01	50.15 ± 3.8	< 0.01
Bumetanide	30.00 ± 4.4	> 0.05	30.47 ± 1.5	> 0.05	40.12 ± 4.2	< 0.01
Furosemide	20.41 ± 4.0	> 0.05	20.91 ± 2.7	> 0.05	20.90 ± 4.5	> 0.05

Values are expressed in mean of seconds ± SD. Acute, subchronic and chronic sertraline and imipramine; subchronic and chronic nifedipine and chronic bumetanide significantly increased swimming times compared to controls. Furosemide had no significant effect on swimming times when compared to control values.

Table 2. Effects of acute, subchronic and chronic sertraline, imipramine, furosemide, nifedipine and bumetanide on climbing times in mice

Drug	Acute	P	Subchronic	P	Chronic	P
Control	30.10 ± 3.5		35.20 ± 2.8		30.65 ± 3.7	
Sertraline	17.49 ± 3.8	< 0.01	15.93 ± 2.0	< 0.01	13.67 ± 4.0	< 0.01
Imipramine	20.08 ± 3.4	> 0.05	30.80 ± 3.0	> 0.05	60.39 ± 3.9	< 0.01
Furosemide	45.00 ± 3.8	< 0.01	45.42 ± 3.0	< 0.01	60.00 ± 3.0	< 0.01
Nifedipine	25.78 ± 1.5	> 0.05	24.15 ± 1.6	> 0.05	24.09 ± 3.4	> 0.05
Bumetanide	35.92 ± 1.8	> 0.05	30.56 ± 2.2	> 0.05	30.61 ± 2.3	> 0.05

Values are expressed in mean of seconds ± SD. Sertraline given acutely, subchronically and chronically had significant effect in decreasing climbing times while nifedipine and bumetanide did not, compared to controls. Acute, subchronic and chronic administration of furosemide and chronic administration of imipramine significantly increased climbing times compared to controls.

when calculated $U_{0.05, 0.01(2)} \geq \text{critical } U$

RESULTS

Effect of sertraline, imipramine, nifedipine, bumetanide and furosemide on swimming times in mice

Effect of the drugs on swimming times in the FST was observed (Table 1) which showed that sertraline significantly ($P < 0.01$) increased swimming time on Day 1. It also significantly ($P < 0.01$) increased swimming times on Day 15 and on Day 31 ($P < 0.01$). Imipramine significantly ($P < 0.01$) increased swimming time on Day 1. It also significantly ($P < 0.01$) increased swimming

times on Day 15 and on Day 31 ($P < 0.01$). Nifedipine did not increase swimming time on Day 1 significantly ($P > 0.05$) but it increased swimming times significantly on Day 15 ($P < 0.01$) and on Day 31 ($P < 0.01$). Bumetanide failed to increase swimming time significantly ($P > 0.05$) on Day 1. On Day 15, bumetanide did not produce increment in swimming time significantly ($P > 0.05$) but did so on Day 31 significantly ($P < 0.01$). Furosemide did not affect swimming time significantly on Day 1 ($P > 0.05$). On Day 15, furosemide did not affect swimming time significantly ($P > 0.05$) and on Day 31, it did not affect swimming time significantly ($P > 0.05$). The swimming action induced by sertraline increases with chronicity of administration while that of imipramine decreases with chronicity of administration.

Effect of sertraline, imipramine, furosemide, nifedipine and bumetanide on climbing times in mice

Effect of the drugs on climbing times in the FST was observed (Table 2) which showed that sertraline significantly ($P < 0.01$) decreased climbing time on Day 1. It also significantly ($P < 0.01$) decreased climbing times on Day 15 and on Day 31 ($P < 0.01$). Imipramine did not significantly ($P > 0.05$) affect climbing time on Day 1. It also did not significantly ($P > 0.05$) affect climbing time on Day 15 but significantly increased climbing time on Day 31 ($P < 0.01$). Nifedipine did not affect climbing times significantly on Day 1 ($P > 0.05$), on Day 15 ($P > 0.05$) and on Day 31 ($P > 0.05$). Bumetanide failed to increase climbing times significantly ($P > 0.05$) on Day 1, on Day 15 ($P > 0.05$) and on Day 31 ($P > 0.05$). Furosemide on the other hand increased climbing times significantly on Day 1 ($P < 0.01$), on Day 15 ($P < 0.01$), and on Day 31 ($P < 0.01$).

DISCUSSION

Pattern of active behaviour with imipramine and sertraline

Previous investigators (Page et al., 1999) have found that sertraline which mediates serotonergic signalling increases swimming time without affecting climbing time and that imipramine which is rapidly converted to desipramine increases climbing behaviour without effecting changes in swimming behaviour. Present results have not agreed with these previous reports probably due to a number of reasons. Steady-state levels of imipramine (which inhibits its own metabolism and is known to affect serotonergic and noradrenergic signal transduction) and its metabolite, desipramine (which mediates noradrenergic signaling) (Detke et al., 1995) varies among individuals mainly due to variation in metabolism and the resultant variation in steady-state concentration may have implications for its effects (Gram, 1988) and this may help explain our findings. Interstrain differences have also been reported by previous investigators (Lopez-Rubalcava and Lucki, 2000) in the response to antidepressant drugs in animal behaviour tests that are sensitive to antidepressants and this may account for sertraline not only affecting serotonergic signaling to cause the increase in swimming times but also significantly causing decrease in climbing times in our experiments. Sertraline may also affect other signal transduction pathways such as the sigma receptor system in complex ways for there is evidence that changes in extracellular serotonin content may result in changes in the regulation of other neurotransmitter systems (Thomas et al., 1998). Sertraline, a sigma receptor antagonist, has been shown to be able to

decrease climbing in the FST (Maj et al., 1996) and its ability to interfere with α_1 -adrenoceptor neurotransmission may also have implications for its effects.

Pattern of Active behaviour induced by Furosemide

Results show that furosemide increases climbing behaviour significantly acutely, subchronically and chronically without significant effect on swimming behaviour. On the other hand, chronic administration of bumetanide exhibited more of swimming behaviour than chronic administration of furosemide (Table 1) showing that it probably interacted more with serotonergic neurotransmission which may find explanation in the differential effect of KCC_2 and $NKCC_1$ on serotonergic signal transduction pathway. Bumetanide is known to only inhibit $NKCC_1$ significantly whereas furosemide significantly inhibits KCC_2 and $NKCC_1$.

Furosemide phosphodiesterase inhibition (Marcus et al., 1978) and adenosine inhibition (O'Connor et al., 1991) could also explain its effect on noradrenergic signaling reflected by the increase in climbing times produced in our experiments. Recently, enhanced expression of KCC_2 was demonstrated in spontaneously depressed Flinders Sensitive Line rats which may be associated with an amplified GABAergic transmission (Matrisciano et al., 2010) and deficiency or under expression of KCC_2 has been noted to be associated with antidepressant-like increased activity in mice (Stil et al., 2011). Furosemide, a KCC_2 blocker (Wang et al., 2006), reduces GABA-evoked responses (Mantovani et al., 2011; Sceniak and Maciver, 2008) and these attributes may contribute to its enhancement of noradrenergic signaling. GABAergic signalling may differentially regulate the noradrenergic and serotonergic systems (Liu et al., 1997)

Pattern of active behaviour exhibited by nifedipine

From our investigations, nifedipine exhibited more of swimming behaviour subchronically and chronically (See Table 1) but not acutely and this confirms previous report that nifedipine interacted with the serotonergic system (Tazi et al., 1992; Mogilnicka et al., 1987). Ansah et al (2008) showed that presynaptic calcium influx is necessary for the actions of the serotonin transporter (SERT) and that the calcium channel blocker, nifedipine, reduced this influx thereby down-regulating the serotonin transporter.

In conclusion, present results suggest that sertraline and imipramine may mediate signal transduction via multiple pathways. Results also suggest that subchronic and chronic administration of nifedipine and chronic adm-

istration of bumetanide mediates serotonergic signaling while acute, subchronic and chronic administration of furosemide mediates noradrenergic signaling. Table 1 and 2

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