Effect of BR-16A on Alpha-2 Adrenergic, Dopamine Autoreceptor and Dopamine Postsynaptic Receptor Functioning

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SUMMARY

BR-16A is a herbal preparation with possible neuropsychiatric effects; preclinical work has found it to facilitate cognition and diminish both anterograde and retrograde amnesia induced by electroconvulsive shocks. The present study sought to assess whether BR-16A affects adrenergic and dopaminergic functioning in the brain. Adult, male, Sprague-Dawley rats which received BR-16A (200 mg/kg/day) or vehicle for one month were challenged with clonidine (100 µg/kg s.c.) apomorphine (2 mg/kg, 100 µg/kg or 50 µg/kg, s.c.) or saline in separate factorial design experiments. Following the challenge, motility of the animals was assessed using a small open field. BR-16A did not influence hypomotility induced by clonidine agonism at alpha-2 adrenergic receptors nor by low dose apomorphine agonism at dopamine autoreceptors. However, BR-16A did augment high dose apomorphine-induced dopamine postsynaptic receptor-mediated hypermotility. These results suggest that BR-16A does not interfere with alpha-2 adrenergic and dopamine autoreceptor functioning, and that it enhances dopamine postsynaptic receptor activity.

Key words: BR-16A; herbal therapy; adrenergic receptor; dopamine autoreceptor; dopamine postsynaptic receptor

BR-16A (Mentat; The Himalaya Drug Company) is a herbal preparation derived from Indian traditional medicine. BR-16A contains Jal-brahmi (Bacopa monnieri) Mandookaparni (Centella asiatica), Ashwagandha (Withania somnifera), Shankapushpi (Evolvulus alsinoides), Jatamansi (Nardostachys jatamansi), Vach (Acorus calamus), Malkangni (Celastrus paniculatus) and Sonth (Zingiber officinale), all of which are claimed to improve memory functions; other ingredients include Tagar (Valeriana Wallachii), Badam (Prunus amygdalus), Salap (Orchis mascula), Lavang (Syzygium aromaticum), Kavach (Mucuna pruriens) and Pearl (Mukta pishti), which are claimed to be nerve tonics; the remaining ingredients are putative general tonics and vitalizers.

An elegant series of preclinical studies has demonstrated that BR-16A improves memory functions. Verma and Kulkarni found that BR-16A reduces scopolamine-induced delay in transfer latency in mice tested in an elevated plus maze.
Kulkarni and Verma\textsuperscript{4} observed that BR-16A attenuates acute and chronic retrograde amnesia induced by electroconvulsive shocks (ECS) in rats tested with a passive avoidance paradigm. Joseph \textit{et al.}\textsuperscript{5} and Andrade \textit{et al.}\textsuperscript{6}, found BR-16A to protect against the development of ECS-induced anterograde amnesia in rats tested in a complex maze and in a ‘T’ maze using a reward oriented paradigm. Faruqi \textit{et al.}\textsuperscript{7}, and Andrade \textit{et al.}\textsuperscript{8}, confirmed the protective effect of BR-16A against ECS-induced anterograde and retrograde amnesia in the complex and T-mazes.

In view of these central nervous system effects of BR-16A, it seems reasonable to expect that the preparation influences neuroreceptor functioning. The present study therefore addressed the possible effects of BR-16A on alpha-2 adrenergic receptor, dopamine autoreceptor and dopamine post-synaptic receptor functioning in the rat brain using behaviourally-assessed neuroreceptor-agonist challenges \textit{in vivo}.

**MATERIALS AND METHODS**

Adult, male, Sprague-Dawley rats, housed 4 per cage with free access to tap water and standard laboratory diet, received either BR-16A (The Himalaya Drug Company) as a fresh aqueous suspension in a dose of 200 mg/kg or vehicle (distilled water) alone. Administration was effected in a volume of 1 ml/kg, once daily for one month. To ensure accurate dosing, BR-16A/vehicle was orally administered by slow syringing through a wide bore, smooth-tipped needle inserted into the posterior part of the pharynx.

The animals were then parenterally challenged with clonidine (Sigma Chemicals; 100 µg/kg), apomorphine (Sigma Chemicals; 2 mg/kg, 100 µg/kg or 50 µg/kg) or saline in separate factorial design experiments with BR-16A/vehicle as one factor and neuroreceptor challenge/saline as the other factor. All challenges were delivered subcutaneously in a volume of 1 ml/kg.

In the administered dose, clonidine is an alpha-2 adrenoceptor agonist, which induces hypomotility; apomorphine is a dopamine receptor agonist, which at low doses induces hypomotility via autoreceptors and at high doses induces hypermotility via post-synaptic receptors\textsuperscript{9-11}.

In effect, therefore, there were four factorial design experiments: one with clonidine for alpha-2 adrenoceptor effects, two with low dose apomorphine for dopamine autoreceptor effects, and one with high dose apomorphine for dopamine postsynaptic receptor effects. In each of the four experiments there were 4 groups: BR-16A + neuroreceptor agonist, BR-16A + saline, vehicle + neuroreceptor agonist and vehicle + saline.

There were 10 rats in each group for each experiment Vehicle-treated rats served as controls to the BR-16A treated group. Saline-injected rats served as internal controls to the neuroreceptor agonist-challenged animals; the internal controls were necessary to control for
perturbations induced by BR-16A in neurotransmitter systems other than that being challenged.

Twenty minutes after the neuroreceptor challenge injection, the animal was placed in a glass cylinder measuring 22 cm in internal diameter and 45 cm in height. Three minutes were allowed for the animal to adapt to the stress of being handled, and to the new environment. Motility of the animal was then recorded as number of quadrants (marked off on the floor of the cylinder) crossed by the animal during a 5-minute period. The procedure followed is the one described for the small open field\textsuperscript{12}. Monitoring was conducted between 11 AM and 3 PM only (to control for diurnal variations in motility) in a sound proof, disturbance-free environment, by an experienced rater who was blind to the experimental status of the rats.

Data were analysed using two way Analysis of Variance. Wherever required, data were first log-transformed to homogenize variances. AP value less than 0.05 was considered significant.

RESULTS

The mean ± SD motility scores of BR-16A and vehicle-treated rats injected with clonidine or with apomorphine are presented in the Table 1.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Challenge</th>
<th>BR-16A</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>Clonidine</td>
<td>0.00 ± 0.00</td>
<td>0.5 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>23.0 ± 4.5</td>
<td>20.8 ± 5.2</td>
</tr>
<tr>
<td>2</td>
<td>Apomorphine (100 µg/kg)</td>
<td>11.9 ± 5.3</td>
<td>8.0 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>10.8 ± 3.6</td>
<td>7.8 ± 6.2</td>
</tr>
<tr>
<td>3**</td>
<td>Apomorphine (50 µg/kg)</td>
<td>6.6 ± 3.0</td>
<td>6.7 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>10.8 ± 3.6</td>
<td>9.8 ± 2.2</td>
</tr>
<tr>
<td>4***</td>
<td>Apomorphine 2 mg/kg</td>
<td>78.4 ± 23.3</td>
<td>29.2 ± 8.4</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>23.0 ± 4.5</td>
<td>20.8 ± 5.2</td>
</tr>
</tbody>
</table>

Values are mean ± SD
*Main effect of clonidine challenge $p<0.001$
**Main effect of apomorphine challenge $p<0.01$
***Main effect of BR-16A vs vehicle $p<0.001$
Main effect of apomorphine challenge $p<0.001$
Challenge x BR-16A/vehicle interaction $p<0.003$
Significances listed above pertain to the indicated experiment; all main/interaction effects not specified were not significant.

There was significant main effect for clonidine ($F_{1,36} = 381.68$, $p<0.001$), indicating that clonidine had induced hypomotility in both groups of rats. The main effect for groups and the group x clonidine challenge interaction were both non-significant, indicating that BR-16A had no independent effect on animal motility, and that it did not alter the hypomotile response induced by clonidine.
With apomorphine challenge in the dose of 100 µg/kg, there was again no significant main effect for groups nor a significant group x challenge interaction. Since the main effect for apomorphine was also nonsignificant, these results were interpreted as an absence of a hypomotile effect of apomorphine due to a failure of unique autoreceptor agonism in the animal model. The experiment was therefore repeated with a lower dose of apomorphine.

With apomorphine challenge in the dose of 50 µg/kg, there was a significant main effect for apomorphine (F1, 36=8.58, p<0.01), indicating that apomorphine had induced hypomotility in both groups of rats. The main effect for groups and the group x apomorphine interaction however remained nonsignificant, indicating that BR-16A had no independent effect on animal motility, and that it did not alter the hypomotile response induced by apomorphine.

With apomorphine challenge in the dose of 2 mg/kg, there was a significant main effect for apomorphine (F1, 36=38.21, p<0.001), indicating that high dose apomorphine had induced hypermotility in both groups of rats. The main effect for groups was also significant (F1, 36=17.92, p<0.001); examination of the data in Table 1 suggest that this result is an artefact of the significant interaction (see below) rather than an independent effect of BR-16A on motility. Most important of all, the group x apomorphine challenge interaction was significant (F1,36=10.36, p<0.003), indicating that there was a greater apomorphine-induced augmentation of motility in BR-16A treated animals than in vehicle treated-animals.

**DISCUSSION**

The general absence of a significant main effect for BR-16A/vehicle groups indicates that BR-16A exerts no effect on animal motility independent of the neuro-transmitter systems challenged. This indicates that BR-16A produces no basal experimental biases with the chemical challenge/animal motility model of neuroreceptor assessment.

Since BR-16A did not alter responses to clonidine challenge, it appears that the preparation does not alter the functioning of alpha-2 adrenergic receptors, the paradigm upon which this model is based.

The failure of apomorphine in the dose of 100 µg/kg to evoke hypomotility is perhaps due to mixed agonism at the high affinity inhibitory autoreceptors and the low affinity facilitatory postsynaptic receptors. A lower dose of apomorphine, 50 µg/kg succeeded in evoking the model; however, since BR-16A did not alter responses to 50 µg/kg of apomorphine, it appears that the preparation does not alter the functioning of dopamine autoreceptors as well, upon which paradigm this model is based.

Since BR-16A augmented the hypermotile response to high dose apomorphine, it appears that the preparation upregulates or sensitizes dopamine postsynaptic receptors, the paradigm upon which this model is based. An implication of this finding is that BR-16A may block dopamine post-synaptic receptors, and that the observed changes in these receptors may
represent a homeostatic effort to overcome or compensate for the chronic receptor blockade. Similar dopamine post-synaptic receptor upregulation is observed following dopamine receptor blockade induced by tricyclic antidepressant and neuroleptic drugs as well\textsuperscript{13}. However, if dopamine receptor blockage is produced by BR-16A, it is not clear why no autoreceptor effects are manifest; as autoreceptor mechanisms exhibit different physiology, this could be an area for further investigation.

While this is the first study to address possible effects of BR-16A on dopamine autoreceptors, the results of these experiments confirm the findings of a previous study\textsuperscript{14} that BR-16A does not influence alpha 3rd adrenoceptor functioning. However, the previous study also reported an absence of influence of BR-16A does not influence of BR-16A on dopamine postsynaptic receptors, in sharp contrast to the findings of the present study. The three possible explanations are:

(i) In the previous study, apomorphine was administered intraperitoneally (as opposed to subcutaneously in this study); opioid alkaloids undergo significant first pass metabolism and hence produce variable and possibly erratic effects when administered by oral or intraperitoneal routes.

(ii) In the previous study, motility was assessed five minutes after the challenge (as opposed to 20 minutes later in the present study), which could have missed the peak chemical challenge effect on the neuroceptors.

(iii) In the previous study, motility monitoring was conducted for a 3 minute span (as opposed to 5 minutes in the present study), which means that random animal behaviour could have had a proportionately greater impact on results.

In summary, BR-16A augments dopamine postynaptic receptor functioning but does not influence functioning of dopamine autoreceptors or alpha-2 adrenergic receptors. It would be premature to speculate upon the implications of these findings and so the clinical significance remains to be determined. Scope for further research includes determination of dose-response curves with neuroreceptor challenge, much as was determined by the preliminary dosage schedules employed in this study. There is also scope to quantity neurochemical changes in the brain, addressing (for example) neurotransmitter molecules, their precursors and their metabolites. Finally, scope exists for the assessment of behavioural effects involving other neurotransmitter systems; towards this goal, the cholinergic and opioid peptidergic effects of BR-16A have already been elegantly studied with positive results\textsuperscript{4,15}.

REFERENCES


