THE EFFECT OF BOVINE FIBRINOPEPTIDES ON THE CENTRAL ACTION OF CHLORPROMAZINE AND AMPHETAMINE IN RATS

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Abstract. A mixture of fibrinopeptides A and B did not evoke any significant central effects when given by intraperitoneal injection, whereas it increased psychomotor activity when injected into a cerebral ventricle. The fibrinopeptides when given by intraperitoneal injection interacted with amphetamine to increase locomotor activity and with chlorpromazine to decrease both locomotor activity and body temperature. It is suggested that the release of fibrinopeptides in various clinical conditions where there is increased fibrinogen-fibrin conversion may lead to an altered sensitivity to centrally acting drugs.

INTRODUCTION

The conversion of fibrinogen to fibrin by thrombin releases two acidic fibrinopeptides A and B (8). These peptides produce physiological changes in concentrations which might be anticipated during the increased fibrinogen-fibrin conversion. Such a situation occurs in the respiratory distress syndrome of the newborn and in some severe bacterial infections (1). In laboratory animals fibrinopeptides have been found to alter pulmonary blood flow, cardiac function (1) and vascular permeability (4). Potentiation of the contractile action of bradykinin on isolated rat uterus by fibrinopeptides has also been reported (8).

Recently we have shown that products of a similar molecular size to fibrinopeptides A and B, produced by tryptic digestion of fibrinogen, cause a marked potentiation of the actions of some centrally acting drugs (2, 3). The aim of this study was to find out whether fibrino-
peptides A and B themselves potentiated the effects of chlorpromazine and amphetamine, since such interactions might be of therapeutic importance.

METHODS

Preparation of fibrinopeptides A and B

Bovine fibrinogen was prepared according to the method of Kekwick et al. (10). A mixture of fibrinopeptides A and B (fAB) was prepared by incubation of an 0.5% fibrinogen solution with 10 NIH units of thrombin (Serum and Vaccine Factory, Lublin) at 37°C according to Osbahr et al. (12).

Capillary permeability test

Male Wistar rats weighing 170–200 g were used for all pharmacological tests. Changes in skin capillary permeability were estimated by comparing the local accumulation of intravenously administered Evans blue dye in the skin after intradermal injection (0.2 ml) of the fAB mixture and after intradermal injections of isotonic saline. Stained spots were eluted with formamide and the concentration of the dye was read photocolorimetrically at 620 nm using elutions from the saline injected spots for a blank reading (13).

Tests for central activity

Body temperature was measured with a thermistor placed in the rectum. Behavior was evaluated by the “open field” test (7) and by Lat’s test (11). In the former test the number of square crossings, rearings, episodes of interest in objects, washing time and defecations were recorded, and in the latter the number of rearings and the time spent walking, washing or the immobile time were recorded. The fibrinopeptides were dissolved in double-distilled water and injected alone or together with drugs, either intraperitoneally or into the right brain ventricle in the volume of 20 μl per rat as described by Herman (9). After the experiments the rats were killed and intraventricular localization of the injected solution was checked by autopsy of the brain. Only those animals which have got correct intraventricular injections were taken under further consideration. The two drugs tested were chlorpromazine, 0.75 mg/kg or 2 mg/kg, and d-amphetamine sulphate 0.5 mg/kg. The level of chlorpromazine in blood and brain tissue was estimated as described by Dubost and Pascal (5).
Statistics

All the experimental data were analysed statistically. The significance of differences was assessed by Duncan's multiple range test, once variances of the experimental groups had been found homogenous by Bartlett-Box test.

RESULTS

Capillary permeability test

The fAB mixture was biologically active, since intradermal injections provoked a dose-dependent increase in skin capillary permeability as measured by the accumulation of Evans blue dye (Fig. 1).

Tests for central activity

The body temperature of untreated rats measured in the rectum was 36.6° ± 0.54°C. No change in body temperature occurred after the intraperitoneal injection of fAB (0.1 mg/kg), whereas chlorpromazine (2 mg/kg) produced an insignificant reduction of body temperature (Fig. 2). When the peptides and chlorpromazine were given together a more marked hypothermic effect was observed. In this case the temperature after 30 min was statistically lower than that in the group which received chlorpromazine alone (P < 0.05).

The fAB mixture did not influence the behavior of animals in either the "open field" or Lat's test (Table I and II). Chlorpromazine, in tested doses, did not produce any statistically significant behavioral change when given alone, whereas amphetamine increased the number of rearings in both tests (P < 0.01) and decreased the time during which the rats remained immobile in Lat’s test (P < 0.01). Marked changes
in the ambulatory behavior of rats were found when the fAB mixture was combined with the drugs. The number of squares crossed ("open field" test) and the time spent walking (Lat's test) were increased approximately two-fold by the combination of fAB mixture with amphetamine and were approximately halved by the combination of fAB mixtures with chlorpromazine ($P < 0.01$). In Lat's test the combination of

Fig. 2. The effect of a mixture of fibrinopeptides A and B on the hypothermic action of chlorpromazine. Each point represents the mean values of 10 experiments. Standard deviations are shown by vertical lines. An asterisk indicates that the rectal temperature of animals receiving the peptides and chlorpromazine was significantly lower than in the group receiving chlorpromazine alone $P < 0.05$.

<table>
<thead>
<tr>
<th>Dose of fAB (μg)</th>
<th>Walking (time in seconds)</th>
<th>Immobility (time in seconds)</th>
<th>Washing (time in seconds)</th>
<th>Number of rearing reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>126±22</td>
<td>409±60</td>
<td>65±27</td>
<td>9±1</td>
</tr>
<tr>
<td>1.0</td>
<td>114±32</td>
<td>399±27</td>
<td>87±25</td>
<td>5±2</td>
</tr>
<tr>
<td>2.0</td>
<td>153±28</td>
<td>366±43</td>
<td>81±41</td>
<td>10±2</td>
</tr>
<tr>
<td>4.0</td>
<td>214±35*</td>
<td>300±40*</td>
<td>86±21</td>
<td>23±3*</td>
</tr>
<tr>
<td>6.0</td>
<td>109±38</td>
<td>403±58</td>
<td>88±24</td>
<td>8±4</td>
</tr>
</tbody>
</table>

* Significantly different from control ($P < 0.01$)
Behavior of rats, evaluated by Lat's test during a 10-min observation period, 30 min following the intraperitoneal injections of a mixture of fibrinopeptides A and B (0.1 mg/kg), amphetamine (0.5 mg/kg) or chlorpromazine (0.75 mg/kg) alone or in combination. Control animals were treated i. p. with saline. The mean values for 10 rats are given with the standard deviations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Walking (time in seconds)</th>
<th>Immobility (time in seconds)</th>
<th>Washing (time in seconds)</th>
<th>Number of rearing reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>131±33</td>
<td>415±35</td>
<td>54±16</td>
<td>7±2</td>
</tr>
<tr>
<td>Fibrinopeptides A and B</td>
<td>114±28</td>
<td>426±54</td>
<td>60±49</td>
<td>10±1</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>185±38</td>
<td>356±32*</td>
<td>59±32</td>
<td>12±2*</td>
</tr>
<tr>
<td>Amphetamine + Fibrinopeptides A and B</td>
<td>232±35*</td>
<td>336±43*</td>
<td>32±28</td>
<td>16±3*</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>91±27</td>
<td>459±45</td>
<td>49±22</td>
<td>5±1</td>
</tr>
<tr>
<td>Chlorpromazine + Fibrinopeptides A and B</td>
<td>54±21*</td>
<td>459±30</td>
<td>87±27</td>
<td>2±1*</td>
</tr>
</tbody>
</table>

* Significantly different from control (P < 0.01)

fAB mixtures with amphetamine further increased the number of rearings produced by amphetamine (P < 0.01) and the combination of fAB with chlorpromazine decreased the number of rearings (P < 0.05). No such changes in the incidence of rearings was observed in the “open field” test.

Table III

Behavior of rats, evaluated by the „open field” test during a 10-min observation period 30 min after intraperitoneal injection of a mixture of fibrinopeptides A and B (0.1 mg/kg), amphetamine (0.5 mg/kg) or chlorpromazine (0.75 mg/kg) alone or in combination. Control animals were treated i. p. with saline. The mean values for 10 rats are given with the standard deviations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of squares</th>
<th>Number of rearings</th>
<th>Number of interest in objects</th>
<th>Washing time in seconds</th>
<th>Number of defecations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41±8</td>
<td>8±2</td>
<td>3±1</td>
<td>44±17</td>
<td>1</td>
</tr>
<tr>
<td>Fibrinopeptides A and B</td>
<td>37±10</td>
<td>5±2</td>
<td>2±1</td>
<td>49±32</td>
<td>0</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>53±8</td>
<td>17±6*</td>
<td>4±2</td>
<td>56±24</td>
<td>1</td>
</tr>
<tr>
<td>Amphetamine + Fibrinopeptides A and B</td>
<td>70±10*</td>
<td>17±5*</td>
<td>4±3</td>
<td>44±24</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>33±6</td>
<td>6±3</td>
<td>3±1</td>
<td>48±28</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpromazine + Fibrinopeptides A and B</td>
<td>22±7*</td>
<td>6±2</td>
<td>3±1</td>
<td>82±47</td>
<td>1</td>
</tr>
</tbody>
</table>

* Significantly different from control (P < 0.01)
When the fAB mixture was injected intraventricularly, dose-dependent behavioral changes were found in Lat’s test (Table III). At the dose of 1 or 2 µg, no consistent variations in any parameter were observed; at the dose of 4 µg, walking, immobility and rearing reactions were significantly increased ($P < 0.01$). However, the highest dose tested (6 µg) was ineffective.

The effect of the fAB mixture on the level of chlorpromazine in blood and brain tissue is shown in Fig. 3. Fifteen minutes after intra-

Fig. 3. The level of chlorpromazine in the blood and brain tissue of rats given a mixture of fibrinopeptides A and B. Each point represents the mean values of 10 experiments. Standard deviations are shown by vertical lines. An asterisk indicates a significant increase in the concentration of chlorpromazine ($P < 0.05$).

peritoneal injection of chlorpromazine the blood level of the drug was statistically higher ($P < 0.05$) in those animals that also received fibrinopeptides, but differences at 30 or 60 min were not significant. The fAB mixture did not significantly change the level of chlorpromazine in brain tissue up to 1 h after injection.

DISCUSSION

Central activity of the fAB mixture after intraventricular injection was demonstrated in Lat’s test but was remarkably dose-dependent. The reasons for the lack of response to the highest dose tested are unclear. The inactivity of the fibrinopeptides after intraperitoneal injection suggests that either they did not readily penetrate into the brain or that critical brain concentrations were not reached to produce behavioral changes. While central stimulatory effects of the fAB mixture might account for its interaction with amphetamine to increase psychomotor activity, they could not explain its interaction with chlorpromazine to inhibit such activity. It was therefore possible that the fibrinopeptides
altered blood or tissue levels of the two drugs. It had previously been shown, for example, that low molecular weight products obtained by trypic digestion of fibrinogen increased the potency of chlorpromazine and amphetamine. The increased activity of chlorpromazine was associated with an increased absorption of the drug from the peritoneal cavity, a decreased binding to plasma protein and an increased accumulation in the brain (3). The increased vascular permeability induced by the fibrinopeptides would support such a hypothesis, since this would increase absorption from the peritoneal cavity. However, while the fAB mixture increased the blood level of chlorpromazine at 15 min, there were no concomitant changes in its concentration in the brain.

Recently Febar and Van Der Meer (6) reported that human fibrinopeptides A potentiated the action of bradykinin. They suggested that the fibrinopeptide influenced the penetration of bradykinin by “opening up” the penetration barriers or by preventing the binding of bradykinin to “silent receptors”. Whether or not the fibrinopeptides used in the present study can modify the actions of chlorpromazine and amphetamine by similar mechanisms, is still to be demonstrated.

Independently of the above considerations, the results obtained could be of some clinical relevance. Bayley et al. (1) have suggested that in pathological states in which fibrinogen-fibrin conversion or deposition may increase, such as thromboembolism, respiratory distress syndrome of the newborn, septicemia, endotoxin shock, oxygen toxicity, and respiratory distress following cardiopulmonary bypass, fAB might accumulate in excessive amounts. Our results indicate that such an accumulation of fAB might markedly potentiate the effects of centrally active drugs.

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