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MODULATORS OF MONOAMINE OXIDASE IN PLASMA

C. T. Giambalvo and R. E. Becker

Rhode Island Psychiatric Research and Training Center, and the Dept. of Pharmacology, Univ. of Rhode Island, Kingston, RI 02881

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Summary

Addition of small amounts of plasma activated the deamination of tryptamine by platelet monoamine oxidase (MAO). At higher concentrations, plasma inhibited the deamination instead. The inhibition was increased with increasing amounts of plasma added. The inhibition was uncompetitive in nature, partially reversed by prior ultrafiltration of the plasma through PM30 membranes and completely reversed by protein precipitation of plasma with perchloric acid. Addition of high amounts of plasma *in vitro* also inhibited the activity of bovine striatal MAO. The inhibition of striatal deamination of tryptamine by plasma was noncompetitive in nature, completely reversed by ultrafiltration through PM30 membranes and partially reversed by perchloric acid treatment. The inhibition of striatal deamination of serotonin was noncompetitive in nature, not reversed by ultrafiltration but completely reversed by perchloric acid treatment. The pattern of inhibition of platelet or striatal MAO by plasma was different from that induced by addition of bovine serum albumin (BSA). Low concentrations of BSA added *in vitro* activated the deamination of tryptamine or serotonin by platelet or striatal MAO by decreasing the K_m, while higher concentrations also decreased the V_{max}. The presence of protein, non-albumin circulating modulators of platelet or striatal MAO in plasma is discussed.

Exposing normal platelets to plasma from chronic schizophrenics has been reported to inhibit platelet monoamine oxidase (MAO) activity while plasma from normal subjects has no effect (1). This finding could not be replicated by Wise et al. (2) who reported that neither plasma from normals or schizophrenics affected MAO activity. Human plasma also has been reported to activate MAO activity (3). We have been studying plasma modulators of MAO in an attempt to link peripheral measures of platelet MAO activity with central nervous tissue MAO activity. We have recently reported that addition of plasma *in vitro* inhibited both platelet and striatal MAO activity, and that the degree of inhibition of platelet MAO was related to the endogenous platelet MAO activity of the individual whose plasma was studied. Moreover, there seemed to be a difference between plasma from normal subjects and schizophrenics in the inhibition of the deamination of 5-HT by human bovine striatal MAO (submitted for publication). Here, we report that these plasma modulators are proteins of various molecular weights. These modulators induced specific changes in the kinetic properties of platelet and striatal MAO that differ from changes with serum albumin.

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Methods

Platelet poor plasma was prepared as follows: venous blood was collected into vacutainers with sodium citrate as anticoagulant. They were centrifuged at 300 x g and 600 x g for 10 minutes respectively to obtain the platelet-rich plasma. The plasma was centrifuged at 1500 x g for 10 minutes. The platelet poor plasma was stored at -40°C until used. The platelet plug was rinsed once in 0.2 N phosphate buffer and then resuspended and frozen. Platelet packs obtained from the blood bank were fractionated in the same way and used as a standard source of MAO for plasma modulator studies. A standard source of striatal MAO was prepared by homogenizing fresh cow striata in 0.32 M sucrose and centrifuging at 1000 x g for 10 minutes to obtain the crude mitrochondrial fraction. The fraction was centrifuged and resuspended in 0.2 N phosphate buffer. It was stored at -40°C until used.

MAO activity was assayed radioenzymatically by the method of Wurtman and Axelrod (4). Briefly, sonicated platelets (containing 300 µg protein) or striatal tissue (containing 30 µg protein) were incubated at 37°C for 30 minutes with ^{14}C-tryptamine (51 mCi/mmole) of various concentrations (from 5 to 50 µM) and adjusted to a final volume of 700 µl with 0.4 N phosphate buffer, pH 7.4. The reaction was terminated with addition of 0.4 ml of 2 N HCl. The indoleacetic acid was extracted into 6 ml of toluene; 4 ml of the extract was counted with 10 ml of Econofluor in the liquid scintillation counter. Platelet MAO activity was determined for each individual whose plasma was used for the modulator study. For the modulator study, 450 µl of crude plasma filtered through millipore filter was added to the standard MAO preparation with varying amounts of ^{14}C-tryptamine in a final volume of 700 µl. MAO activity was then assayed as described above. To measure the deamination of 5-HT, striatal tissue (containing 75 µg protein) were incubated with ^{14}C-5-HT (51 mCi/mmole) of varying concentrations, 50 - 500 µM, at 37°C for 30 minutes. The 5-hydroxy-indoleacetic acid formed was extracted into 6 ml of toluene: ethylacetate (1:1); 4 ml of the extract was counted with 10 ml of Econofluor in the liquid scintillation counter. The kinetic analysis was performed on the computer, using the method of Wilkinson (5). Protein was determined by the Lowry method (6).

Aliquots of crude plasma were ultrafiltered through PM30 Amicon membranes to remove substances greater than 30,000 MW. The ultrafiltered plasma was then added *in vitro* to MAO preparations. Aliquots of plasma were also treated with 0.4 N perchloric acid to precipitate plasma proteins. The perchloric acid extract was then neutralized with KOH at 0°C and centrifuged to remove the $KClO_4$ salt. The supernatant was then added to tissue with buffer for MAO determination. Results of the pretreatments were compared with untreated plasma by paired t-test.

Results

The effects of adding varying amounts of plasma to platelets on MAO activity is shown in Fig. 1. Low concentrations of crude plasma (less than 100 µl) slightly activated MAO while high concentrations of plasma inhibited MAO in a linear fashion. The inhibition seen with the higher concentrations was uncompetitive in nature, with a parallel decrease in the K_m and V_{max} (52%) (Fig. 2, Table 1). Prior precipitation of plasma proteins with perchloric acid completely reversed the inhibition (Table 1), suggesting that the inhibitor contained protein moieties. After ultrafiltration of the crude plasma through PM30 Amicon membranes to remove substances > 30,000 molecular weight, significant inhibitory activity persisted (Fig. 2, Table 1). This suggests that the bulk of the inhibitory activity can be accounted for by substances < 30,000 MW. This also suggests that the inhibitory substance is unlikely to be serum albumin which has a molecular weight of 67,000. As further evidence that inhibition was not mediated by serum albumin, various concentrations of bovine serum