Age and sex differences related to platelet aggregation

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Johnson et al. (1975) in reporting their results on platelet response to aggregating agents concluded that there is an age and sex difference. In their study, they tried to establish a relationship between platelet responsiveness and the tendency of men to show higher mortality to cardiovascular disease than women. The studies of Johnson (1975) showed that male platelet responsiveness increased significantly with age and they related this to the 'high-risk' cardiovascular disease groups.

The present experiments were carried out to reinvestigate age and sex differences in platelet response to aggregating agents. We used ADP, an aggregating agent with a variable response depending on concentration, producing either reversible (0.5μm) with marked disaggregation or irreversible (1.0μm) aggregation (very little, if any, disaggregation), and arachidonic acid, an irreversible aggregating agent (75μm). Preliminary experiments were done to determine the concentrations of these agents necessary to produce the required response.

Blood samples were taken from the cubital vein of volunteers into plastic syringes and immediately carefully mixed in all plastic containers containing sodium citrate as anti-coagulant (0.38% final concn.). None of the volunteers had taken recent medication, especially salicylates. Samples from females were taken without reference to the menstrual cycle. All samples were taken between 08:30 and 09:30h and aggregation was determined no later than 1h after sampling. Platelet-rich (PRP) and platelet-poor plasmas (PPP) were prepared, and platelet counts were determined no later than 1h after sampling. Platelet-poor plasmas (PPP) were prepared, and platelet counts were determined no later than 1h after sampling. Platelet-rich (PRP) and platelet-poor plasmas (PPP) were prepared, and platelet counts were determined no later than 1h after sampling.

Fig. 1. Maximum aggregation induced by ADP (a) and arachidonic acid (b) in human platelets

Values are expressed as percentage changes in transmission when the difference between platelet-poor and platelet-rich plasma was adjusted to 100%. The challenge was either 0.5μm ADP or 75μm arachidonic acid in iso-osmotic Tris/HCl buffer, pH 7.4. Additions of buffer solutions and control buffers to platelet-rich plasma were in the ratio plasma/buffer 10:1. Incubations were at 37°C.

Significance of results: males versus females: ADP: group A P<0.01; group B P=0.2-0.05 (N.S.); group C P=0.7 (N.S.); arachidonate: group A P=0.1-0.05 (N.S.); group B P=0.1-0.05 (N.S.); group C P=0.2 (N.S.). ADP: male groups A versus C, P=0.05; female groups A versus C, P=0.2 (N.S.). Arachidonate: male and female groups A versus C, no significant differences.
3.5 ± 0.60 (7), 26–35 years, 3.6 ± 0.47 (5), 36 + years 3.6 ± 0.63 (10). No significant differences were found between age groups or males and females. Aggregation patterns are shown in Fig. 1. 36 + refers to subjects 36 years and over; the oldest male was 60 years, and the oldest female 58 years.

Maximum aggregation response induced by both 0.5 μM- and 1.0 μM-ADP in the young group (15–25 years, mean 18.8 years) was significantly greater (P < 0.01) in females than in males, at least twice as high. When arachidonic acid was used as the aggregating stimulus, both the 15–25 year and 26–35 year female group showed a higher mean value than males, but the results were barely significant (P = 0.10–0.05). The results for aggregation agree with previous reports (Zahavi et al., 1973; Johnson et al., 1975) that platelets from females have a higher sensitivity than those from males to aggregation by ADP.

Disaggregation measured 5 min after challenge with 0.5 μM-ADP was greater (2 ×) in the young male (15–25 year age group) than corresponding females (P = 0.05). At the low concentration of arachidonic acid used, we obtained some degree of disaggregation, and again this was more marked in the 15–25 year male group (P = 0.05–0.02). Disaggregation after ADP treatment did not differ greatly with increase in age, whereas after treatment with arachidonate, an irreversible aggregator, disaggregation decreased very markedly with increase in age in males, in contrast with females.

There was a steady increase in platelet sensitivity to aggregating stimuli with increase in age in males, but not in females.

The increase in males from the 15–25-year group to the 36 + year group was significant at the P = 0.05 level (0.5 μM-ADP). The lack of increasing sensitivity in females in our groups contrasts with the findings of Johnson et al. (1975).

The increased platelet sensitivity to ADP in males has been quoted as one of the causes of increased risk to cardiovascular disease over 40 years of age. It would appear, however, that this cannot be a major causative factor, since females show a steady level of response throughout life, and the 'female' response is just as high, and in fact, higher in most cases than that of males of 36 years and over, i.e. in the high-risk group. However, it should be noted that we found that disaggregation after treatment with arachidonate, a powerful irreversible aggregating agent, was markedly reduced with increase in age in males and not in females. The facility of platelets to disaggregate may be more important than the sensitivity to aggregate for the avoidance of thrombus formation in the circulation.

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**[3H]Spiroperidol binding in bovine caudate nucleus**

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In recent years [3H]spiroperidol binding has been investigated in a variety of species and brain regions. In the striatum, it is thought that [3H]spiroperidol primarily labels dopamine receptors (see, for example, Fields et al., 1977), and much work has been carried out using this ligand because of its apparent high affinity for the dopamine receptor. However, it appears that [3H]spiroperidol can also label serotonin (5-hydroxytryptamine) receptors (Creese & Snyder, 1978), although the binding of [3H]spiroperidol to serotonin receptors in the striatum is low compared with its binding to dopamine receptors (Quik & Iversen, 1979). The present communication describes preliminary experiments conducted to obtain a more complete analysis of [3H]spiroperidol binding in caudate nucleus, a major component of the striatum.

A microsomal fraction from bovine caudate nucleus was obtained as described previously (Withy et al., 1979) and used in these studies. [3H]spiroperidol binding was measured by a modification of the procedure described by Howlett & Nahorski (1978), using a protein concentration of 0.1 mg/ml, and a physiological medium (pH 7.4) similar to that used by Strange et al. (1978), but containing Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] as buffering agent.

The nature of [3H]spiroperidol binding was investigated by the use of mianserin, a potent serotonin-receptor antagonist (Vargaftig et al., 1971). Fig. 1 shows the displacement of total [3H]spiroperidol (0.25 μM) binding by this drug. The displacement curve is biphasic and the data can be fitted to a model where displacement occurs at two independent sites (see Fig. 1): 28% of the binding is displaced with an IC50 value of 6 nM, and 72% of the binding is displaced with an IC50 of 10 μM. It is likely that the binding sites showing a high affinity for mianserin (IC50 = 6 nM) are serotonin-binding sites, whereas the sites showing a low affinity (IC50 = 10 μM) are dopamine-binding sites. This analysis agrees with the data of Leysen et al. (1978) for the inhibition of [3H]spiroperidol binding by mianserin in the striatum and frontal cortex, and shows that in the striatum [3H]spiroperidol labels serotonin-binding sites as well as dopamine-binding sites.

These results also indicate that a concentration of 0.1 μM-mianserin can be used to inhibit binding of [3H]spiroperidol to...