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Effects of methysergide on platelets incubated with reserpine

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Summary

1. Platelets were incubated with methysergide and related compounds (2-bromo lysergic acid (BOL), ergotamine and methyl ergotamine) together with reserpine.

2. Methysergide inhibited the normal aggregation response of platelets to 5-hydroxytryptamine (5HT) but did not affect the reduction in the 5HT content caused by reserpine, or the uptake of 5HT by the platelets.

3. BOL, ergotamine and methyl ergotamine behaved similarly. Methysergide had greater anti5HT potency than BOL, and methyl ergotamine had greater potency than ergotamine.

4. The use of platelets as a model for synaptic preparations is discussed.

5. The role of 5HT receptor sites on the platelet membrane and the significance of the results for migraine patients treated with methysergide are discussed.

Introduction

Platelet aggregation responses to 5-hydroxytryptamine (5HT) are strongly inhibited after *in vitro* preincubation with methysergide (O'Brien, 1964; Hilton & Cumings, 1971).

Baumgartner & Born (1968) have suggested that 5HT-induced aggregation is a measure of the availability of uptake sites for the transfer of 5HT through the platelet membrane. This paper reports on the effect of methysergide on the uptake of 5HT into platelets and its release from platelets, using aggregation responses to 5HT and incubation experiments in which the 5HT content of platelet samples was determined.

Methysergide is used as a prophylactic treatment for migraine; reserpine, which depletes 5HT from body stores, can induce migraine-like headaches in migraine sufferers (Kimball & Friedman, 1961). In the experiments to be described, platelets from control subjects were used as a convenient model to study the action on 5HT stores of methysergide and reserpine together.

Methods

Samples of human blood were obtained from normal control subjects using disposable syringes and siliconized glassware throughout, and the platelet aggregation tests were carried out as described previously (Hilton & Cumings, 1971) using

an EEL platelet aggregation meter linked to a Honeywell chart recorder. Two preincubation agents, for example reserpine and methysergide (0·05 ml each), were added at consecutive short intervals to the plasma (1 ml), kept at 37° C, before testing the aggregation induced by 5HT.

Blood for the incubation experiments and the estimation of 5HT was collected into 0·5% sodium ethylene diamine tetra-acetate (EDTA) dissolved in a 0·9% w/v aqueous solution of sodium chloride. (Blood, 4 volumes: EDTA in NaCl solution, 1 volume). Platelet rich plasma was obtained within 1 h of blood collection by centrifuging at 700–800 r.p.m. for about 10 minutes. Three 1 ml samples were pipetted into siliconed conical glass test-tubes and the incubations started within 2 hours. The plasma samples were warmed in a water bath at 37° C for about 5 min before adding 0·1 ml amounts of 0·9% w/v NaCl solution or the incubation agents. The tubes were tapped gently to effect mixing and replaced in the water bath for the incubation time.

In most instances two incubation agents were used, to see if they enhanced or reduced each other's effect. Plasma sample 1 was used as a control to which 0·9% w/v NaCl solution was added in place of each incubation agent; to sample 2, 0·9% w/v NaCl solution was added in place of the first agent; plasma sample 3 was used as the test experiment and both agents were added. In 5HT depletion experiments, the plasma was incubated with the first agent (methysergide, $4{\cdot}3\times10^{-9}$ mol; 2-bromolysergic acid (BOL), $4{\cdot}3\times10^{-9}$ mol; ergotamine, $1{\cdot}5\times10^{-9}$ mol, or methyl ergotamine, $1{\cdot}5\times10^{-9}$ mol, each in 0·1 ml) for 5 min followed by reserpine (2×10^{-9} mol in 0·1 ml) for 30–60 min incubation. In 5HT uptake experiments, the plasma was incubated with the first agent (in strength and volume as above) for 5 min, followed by 5HT (5×10^{-8} mol in 0·1 ml) for 30–60 min incubation. In a few cases methysergide ($4{\cdot}3\times10^{-9}$ mol in 0·05 ml) and reserpine (2×10^{-9} mol in 0·05 mol) were added together for the first incubation of 5 min, before 5HT (5×10^{-8} mol in 0·1 ml) was added for the second incubation.

At the end of the incubations the tubes were removed from the water bath and 4 ml of 0·9% w/v NaCl solution was pipetted into each. The tubes were then centrifuged at about 1,500 r.p.m. for 12 min and the supernatant liquid gently drained off the platelet button. The inside of each tube was dried carefully and 2 ml 0·9% w/v NaCl solution was added by pipetting down the side to avoid disturbing the platelet button which was rinsed by slowly turning the tubes. The NaCl solution was decanted off, the inside of the tube again dried and 2·5 ml 0·9% w/v NaCl solution added. The platelets were resuspended by mixing on a Rota mixer, and the resulting suspensions were frozen and could then be kept at −20° C for up to 1 week before estimations of 5HT were made. The contents of the tube were thawed and frozen again to ensure complete lysis of the cells, before the determination of 5HT was carried out, using the method of Ashcroft, Crawford, Binns & McDougall (1964).

Materials

The following solutions in 0·9% w/v NaCl solution were prepared daily to contain the amount shown for each portion (0·05 ml or 0·1 ml) used, stored at 40° C and added to 1 ml plasma: 5HT (serotonin creatinine sulphate, BDH), 5×10^{-8} mol; reserpine (2·5 mg vial. Boots), 2×10^{-9} mol; methysergide maleate (Sandoz).

$4{\cdot}3 \times 10^{-9}$ mol; BOL (2-bromo lysergic acid) (Sandoz), $4{\cdot}3 \times 10^{-9}$ mol; ergotamine tartrate (Sandoz), (a) $4{\cdot}3 \times 10^{-9}$ mol, (b) $1{\cdot}5 \times 10^{-9}$ mol; methyl ergotamine tartrate (Sandoz), (a) $4{\cdot}3 \times 10^{-9}$ mol, (b) $1{\cdot}5 \times 10^{-9}$ mol.

The ratios of reserpine (2×10^{-9} mol) to methysergide ($4{\cdot}3 \times 10^{-9}$ mol) to ergotamine ($1{\cdot}5 \times 10^{-9}$ mol) are in the same proportion as the doses used clinically, that is reserpine (2·5 mg) to methysergide (4 mg) to ergotamine (4 mg). Reserpine (2·5 mg, i.v.) will induce migraine-like headaches in migraine sufferers (Kimball & Friedman, 1961) and clinical doses of methysergide (4 mg) and ergotamine (4 mg) are used in the treatment of migraine.

Measurement of results

The aggregation of the platelets in plasma was measured as the maximum rate of decrease of optical density, measured on the chart paper. Platelet aggregation values, using plasma (of control subjects) preincubated with various drugs, were expressed as described previously (Hilton & Cumings, 1971). The aggregation response to 5HT after preincubation was expressed relative to the response without preincubation, in order to distinguish the effect of preincubation from the latent response to 5HT, that is the results were expressed in terms of R_i/R where: R_i represents the rate of aggregation after preincubation, and R represents the rate of aggregation without preincubation. Thus a value of 1·0 for R_i/R infers that no change in the aggregation response has occurred as the result of preincubation; a value greater than 1 shows potentiation of the response and less than 1, inhibition.

Results for the content of 5HT are expressed as the 5HT content of the platelet button in the test relative to that of the control platelet button which had been incubated with 0·9% NaCl solution only.

Results

Plasma was incubated with reserpine followed by methysergide and the aggregation response to 5HT measured. The reserpine preincubation time of 30 s was sufficient to remove the strong inhibition found with a short period of methysergide preincubation, as can be seen in Table 1. Longer preincubation with methysergide in the presence of reserpine reduced the aggregation response to values comparable to those obtained using methysergide alone.

TABLE 1. *Effect of reserpine on the methysergide preincubation of platelets before aggregation with 5HT*

Time of reserpine preincubation	Time of methysergide preincubation	Aggregation after preincubation $R_i/R \pm$S.E.M.
30 s	5 s	1·10±0·06 (12)
30 s	30 s	0·54±0·04 (12)
30 s	2 min	0·15±0·04 (12)
Not used	5 s	0·54±0·11* (12)
Not used	2 min	0·17±0·02 (6)

The number of observations contributing to each result is shown in parentheses. *See Hilton & Cumings (1971). The amounts of reserpine and methysergide present in 1 ml of incubation mixture were 2×10^{-9} mol and $4{\cdot}3 \times 10^{-9}$ mol respectively.

Similar experiments were performed, with an initial incubation of methysergide, followed by reserpine. The aggregation response was substantially reduced and did not approach that observed after reserpine alone, as can be seen in Table 2.

The 5HT content of platelet samples incubated with reserpine alone and with methysergide followed by reserpine was determined and expressed relative to the 5HT content of the platelets incubated with saline. Reserpine reduced the 5HT content to 75% of its initial level within 30 min and no further decrease was noted in 1 h, as can be seen in Table 3 (a). The methysergide incubation had no effect on the reserpine depletion.

The effect of an initial incubation of reserpine on the uptake of 5HT was studied and the results can be seen in Table 3 (b). The exact quantity of 5HT taken up was variable between platelets from different subjects and hence the standard errors of the mean were larger than for depletion experiments. Reserpine reduced the uptake of 5HT to 52% and 57% of that in saline controls, after incubations lasting 30 and 60 minutes.

The effect of methysergide on the uptake of 5HT was investigated, performing similar experiments and incubating first with methygerside, then with 5HT. The methysergide had no effect on the uptake, as can be seen in Table 3 (c).

To test the effect of methysergide and reserpine together on 5HT uptake, methysergide was added with the reserpine to plasma samples which were incubated for 5 min before adding 5HT. The 5HT content of the platelet samples was reduced to about 40% of that of the samples incubated initially with saline, as can be seen in Table 3 (d). This reduction in 5HT content (44%, 44%, 52%, 43%) is not significantly different from that after reserpine alone (52%, 57%) shown in Table 3 (b).

The effects of BOL, ergotamine and methyl ergotamine on the depletion of 5HT by reserpine and on 5HT uptake had no statistically significant effect on the release or uptake of 5HT.

The results of preincubating platelets with ergotamine and methyl ergotamine before testing the aggregation response to 5HT are shown in Table 4 where they are compared with results for methysergide, using concentrations in proportion to their therapeutic dosages. All three compounds inhibited 5HT-induced aggregation, as

TABLE 2. *Effect of methysergide on the reserpine preincubation of platelets before aggregation with 5HT*

Time of methysergide preincubation	Time of reserpine preincubation	Aggregation after preincubation $R_i/R \pm$ S.E.M.
5 s	30 s	0·36±0·03 (12)
30 s	30 s	0·42±0·03 (12)
2 min	30 s	0·32±0·03 (12)
30 s	5 min	0·34±0·05 (12)
Not used	30 s	0·98±0·11* (12)
Not used	5 min	0·81±0·11* (12)

The number of observations contributing to each result is shown in parentheses. *See Hilton & Cumings (1971). The amounts of reserpine and methysergide present in 1·2 ml of incubation mixture were 2×10^{-9} mol and $4{\cdot}3 \times 10^{-9}$ mol, respectively.

shown in the first three columns. Methyl ergotamine is a stronger inhibitor than ergotamine, but neither is as effective as methysergide.

The inhibition of aggregation by methysergide, BOL, ergotamine and methyl ergotamine in equimolar concentrations can be seen in Table 4 in the last three columns together with the first column. Methysergide is the strongest inhibitor and reduces the aggregation response even after 5 s, and BOL shows a similar but reduced effect. After preincubation for 1 min methysergide is a stronger inhibitor than BOL and methyl ergotamine is a stronger inhibitor than ergotamine.

TABLE 3 (*a*). *Effect of methysergide on the depletion of 5HT by reserpine*

First incubation of 5 min with:	0·9% $^{w}/_{v}$ NaCl solution	0·9% $^{w}/_{v}$ NaCl solution	Methysergide	
Second incubation with:	0·9% $^{w}/_{v}$ NaCl solution	Reserpine	Reserpine	
Time (min)		5HT ratios†		% difference in depletion
30	1 (5)	0·75±0·02 (5)	0·73±0·02 (5)	n.s.
60	1 (5)	0·76±0·06 (5)	0·74±0·07 (5)	n.s.

(*b*). *Effect of reserpine on the uptake of 5HT*

First incubation of 5 min with:	0·9% $^{w}/_{v}$ NaCl solution	0·9% $^{w}/_{v}$ NaCl solution	Reserpine	
Second incubation with:	0·9% $^{w}/_{v}$ NaCl solution	5HT	5HT	% reduction of uptake
Time (min)		5HT ratios†		
30	1 (5)	2·9±0·2 (5)	1·5±0·1 (5)	52
60	1 (5)	4·9±0·6 (5)	2·8±0·7 (5)	57

(*c*). *Effect of methysergide on the uptake of 5HT*

First incubation of 5 min with:	0·9% $^{w}/_{v}$ NaCl solution	0·9% $^{w}/_{v}$ NaCl solution	Methysergide	
Second incubation with:	0·9% $^{w}/_{v}$ NaCl solution	5HT	5HT	% reduction of uptake
Time (min)		5HT ratios†		
30	1 (5)	4·7±0·6 (5)	4·5±0·4 (5)	n.s.
60	1 (5)	5·4±0·5 (5)	5·4±0·5 (5)	n.s.

(*d*). *Effect of methysergide and reserpine on the uptake of 5HT*

First incubation of 5 min with:	0·9% $^{w}/_{v}$ NaCl solution	0·9% $^{w}/_{v}$ NaCl solution	Methysergide and reserpine	
Second incubation with:	0·9% $^{w}/_{v}$ NaCl solution	5HT	5HT	% reduction of uptake
Time (min)		5HT ratios†		
30	1 (5)	4·0±0·2 (5)	1·8±0·3 (5)	44
60	1 (5)	5·0±0·8 (5)	2·2±0·8 (5)	44
30	1 (5)	3·5±0·4 (5)	1·9±0·3* (5)	52
60	1 (5)	4·3±0·1 (5)	1·9±0·3* (5)	43

The amounts of reserpine, methysergide and 5HT present in 1·2 ml of incubation mixture in all experiments were 2×10^{-9} mol, $4{\cdot}3\times10^{-9}$ mol and 5×10^{-8} mol, respectively except for those marked * where the amount of methysergide was $8{\cdot}6\times10^{-9}$ mol. Each result is shown ±standard error of the mean. The number of observations contributing to each mean is shown in parenthesis. n.s. represents not significant. †The 5HT in the platelets is expressed as the ratio of the 5HT content of the platelets in the test to the 5HT content of the platelets incubated with 0·9% $^{w}/_{v}$ NaCl solution.

TABLE 4. *Effect of methysergide and related compounds on platelet aggregation induced by 5HT*

Pre-incubation with:	Aggregation ($R_1/R \pm$S.E.M.) Methysergide maleate*	Ergotamine tartrate	Methyl ergotamine tartrate	BOL	Ergotamine tartrate	Methyl ergotamine tartrate
Amount present in incubation mixture	$4{\cdot}3\times10^{-9}$ mol	$1{\cdot}5\times10^{-9}$ mol	$1{\cdot}5\times10^{-9}$ mol	$4{\cdot}3\times10^{-9}$ mol	$4{\cdot}3\times10^{-9}$ mol	$4{\cdot}3\times10^{-9}$ mol
Preincubation time						
55	$0{\cdot}54\pm0{\cdot}11$ (12)	$1{\cdot}03\pm0{\cdot}12$ (6)	$1{\cdot}05\pm0{\cdot}04$ (6)	$0{\cdot}79\pm0{\cdot}03$ (6)	$1{\cdot}03\pm0{\cdot}06$ (6)	$1{\cdot}10\pm0{\cdot}05$ (6)
1 min	$0{\cdot}13\pm0{\cdot}05$ (12)	$0{\cdot}83\pm0{\cdot}09$ (6)	$0{\cdot}65\pm0{\cdot}13$ (6)	$0{\cdot}33\pm0{\cdot}07$ (12)	$0{\cdot}65\pm0{\cdot}08$ (6)	$0{\cdot}25\pm0{\cdot}06$ (6)
5 min	$0{\cdot}05\pm0{\cdot}12$ (6)	$0{\cdot}63\pm0{\cdot}11$ (6)	$0{\cdot}18\pm0{\cdot}05$ (6)	$0{\cdot}17\pm0{\cdot}07$ (12)	$0{\cdot}07\pm0{\cdot}04$ (6)	$0{\cdot}07\pm0{\cdot}06$ (6)

The number of observations contributing to each result is shown in parenthesis. *See Hilton & Cumings (1971).

Discussion

The results presented here show that methysergide, BOL, ergotamine and methyl ergotamine inhibit, to varying degrees, 5HT-induced platelet aggregation, but have no effect on the uptake of 5HT into the platelets or the release of 5HT by reserpine from the platelets. Baumgartner & Born (1968) consider that the 5HT-induced aggregation of platelets is dependent on the availability of uptake sites on the platelets. When the uptake site is empty, aggregation can occur but if the uptake site is already occupied by a 5HT molecule, aggregation cannot take place.

Thus the platelet aggregation results might be explained by suggesting that a 5HT antagonist, for example methysergide, also occupies an uptake site on the platelet membrane, which is thereby rendered unavailable for 5HT and hence for aggregation. Since the antagonist occupies the receptor site, the uptake and release of 5HT should be impaired; however, no such effect was found in these experiments (see Table 3).

All the compounds tested here have anti5HT activity. Using the platelet aggregation tests, methysergide is a stronger 5HT antagonist than BOL, and methyl ergotamine a stronger antagonist than ergotamine. In the literature LSD (D-lysergic acid diethylamide tartrate) is the best documented 5HT antagonist and much work relates to its effects. For example, it has been reported that BOL and LSD have similar potencies on isolated organ preparations and that methysergide is more potent that LSD in the rat paw oedema test (Gyermek, 1961). The anti5HT potencies of methysergide and BOL on rat uterus preparations have been given as 600–700% and 70–150%, respectively, taking LSD activity to be 100% (Offermeier & Ariëns, 1966). Members of the ergotamine group are weaker antagonists on rat uterus, showing only 3–11% of the potency of LSD, but have potencies approaching that of LSD on rabbit ear preparations (Gyermek, 1961). However, ergotamine has 6·7% and methyl ergotamine 13·2% activity on rat paw oedema, taking LSD to have 100% activity (Doepfner & Cerletti, 1958). More recently, the ability of LSD, BOL and methysergide to antagonize the action of 5HT applied to single neurones in the brain stem of decerebrate cats by microiontophoresis, has been studied by Boakes, Bradley, Briggs & Dray (1970) who found that LSD antagonized the excitatory actions of 5HT in thirty-one out of thirty-two neurones, methysergide affected twenty-three out of forty-seven neurones and BOL only six out of thirty-three neurones tested.

The platelet aggregation results presented in this paper are interesting in the light of claims made by Page (1968) and Pletscher (1968) that platelets might disclose similarities with synaptic vesicles and provide a more available model of 5HT function and transfer than synaptic vesicles themselves. Carnegie (1971) has recently identified the amino-acid sequence of the suggested 5HT binding site in synaptosomal membranes and this binding site has the characteristics suggested for a possible 5HT receptor site on the neuronal membrane (Smythies, Benington & Morin, 1970). It is thought that an antagonist (or false transmitter) could interact with such a receptor site, preventing the transmitter from activating it and therefore depressing the effects of the released physiological transmitter, or the false transmitter may activate the receptor but not as efficiently as the transmitter itself (Kopin, 1970). LSD might act at a receptor on the raphe nuclei neurones or have a 5HT-like effect at a postsynaptic site (Aghajanian, 1970). This might activate a negative

feedback circuit and result in an inhibition of raphe nuclei cells. BOL had a similar but reduced antagonistic effect.

The 5HT antagonists have no effect on the 5HT content of platelets after incubation with 5HT or reserpine. Aghajanian (1970) considered that inhibition by LSD and BOL in the central nervous system would result in an increase in 5HT content but a decrease in the content of its major metabolite 5-hydroxyindoleacetic acid (5HIAA). Increased urinary concentrations of 5HIAA have been noted in subjects on a prophylactic dose of Deseril (methysergide) (Curran, Hinterberger & Lance, 1965; Pokora, 1966). However, the mean blood 5HT content in patients with migraine, taking methysergide prophylactically, is not significantly different from the 5HT content of patients not on drugs (Curran, Hinterberger & Lance, 1965; Hilton & Cumings, 1971), which might suggest an increased turnover of 5HT in patients on methysergide.

Methysergide is a very effective prophylactic treatment for migraine (Sicuteri, 1963; Graham, 1964; Lance, Anthony & Somerville, 1970) and its mechanism of action may be at 5HT binding sites (such as discussed here) located on neuronal surfaces, on platelets, and also in the vascular bed. Walsh (1967) concluded that in vessels of the upper limbs, 5HT acts on specific receptors in the vascular system which are independent of catecholamine and acetylcholine receptors and that methysergide can antagonize the effects of 5HT on these receptors. In the past it has frequently been remarked that it is strange that a 5HT antagonist should prove so useful in controlling migraine, for during an attack the blood 5HT concentration drops sharply (Curran, Hinterberger & Lance, 1965). It is possible that in mimicking the effect of 5HT on receptors methysergide circumvents potential headaches. On the other hand, methysergide may prevent the concentration of 5HT in the blood from rising too high—and an initially high 5HT concentration (vasoconstrictor phase) before the drop in the concentration of 5HT (vasodilatation phase) has been implicated in the pathogenesis of migraine (Anthony, Hinterberger & Lance, 1967).

The effectiveness of ergotamine in relieving migraine attacks is usually attributed to its capacity to constrict smooth muscle of arterial walls and relieve the vasodilatation which often accompanies the painful phase of the headache (Graham, 1956). It is possible that the vascular effects of ergotamine are due to interactions with 5HT binding sites and the mechanism is like that outlined for methysergide above. Ergotamine may also have similar effects on platelets and neuronal membranes and these effects could contribute to its therapeutic action. Little work has been done on the effect of ergotamine on 5HT metabolism. Farris, Rapelli & Mathis (1967) found reductions in the urine 5HIAA concentrations in normal control subjects 1 h after ergotamine tartrate (0·5 mg i.v.). This result was not in agreement with the rise in 5HIAA anticipated to occur in rat brain by Aghajanian (1970) following LSD and BOL, but 1 h is perhaps too short an interval to expect changes in a urinary metabolite.

The experiments performed were intended to give information about the action of methygerside and its related compounds on platelet aggregation and 5HT receptor sites and also to be a model for the possible *in vivo* situation where a migraine patient on a prophylactic dose of methysergide is given reserpine, to deplete body stores of 5HT. The results would indicate that the platelets from such a patient would have low aggregation responses due to the action of the 5HT antagonist,

but the reduction in platelet 5HT due to reserpine would not be altered. Thus under these conditions if a headache develops in a migraine patient, changes in 5HT concentration could be the initiating factor. If no headache develops, and as no information on this subject is as yet available, the role of methysergide on 5HT receptor sites requires investigation.

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