Platelet Aggregability in Sleep-Related Stroke

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ABSTRACT: We examined platelet aggregability during nocturnal sleep and daytime wakefulness in patients with a history of sleep-related stroke onset (SOS) and compared it to that of matched awake-onset stroke (AOS) patients and controls without evidence of vascular disease. Aggregability was evaluated *in-vitro* at least seven weeks following stroke onset. Platelets were more aggregable to ADP, collagen and arachidonic acid (AA) during both sleep and wakefulness in patients with AOS ($p<0.01$). No significant difference in the mean aggregation thresholds during sleeping or waking periods were found between SOS and control groups. However, platelets were significantly more responsive to AA during sleep than during wakefulness in the SOS patients ($p<0.01$). This difference was confined to the subgroup of SOS patients who had experienced nocturnal as opposed to daytime sleep-related stroke onset, suggesting that the observed difference in platelet responsiveness to AA may be related to a circadian fluctuation in platelet aggregability rather than to a sleep-related fluctuation. Significant sleep-related changes in platelet aggregability were not identified in the other two groups.

RÉSUMÉ: Agrégation plaquettaire dans les accidents cérébrovasculaires survenant pendant le sommeil Nous avons étudié l'agrégation plaquettaire pendant le sommeil nocturne et la période de veille diurne chez les patients ayant une histoire d'accident cérébrovasculaire (ACV) dont le début était relié au sommeil et nous avons comparé ces données à celles recueillies chez des patients appariés dont l'ACV avait débuté pendant l'état de veille et chez des contrôles sans évidence de maladie vasculaire. L'agrégation plaquettaire a été évaluée in-vitro au moins sept semaines après la survenue de l'ACV. L'agrégation des plaquettes à l'ADP, au collagène et à l'acide arachidonique (AA) était plus marguée pendant le sommeil et pendant l'état de veille chez les patients dont l'ACV avait débuté alors que le patient était en état de veille (p <0.01). Nous n'avons pas observé de différence significative entre le seuil moyen d'agrégation pendant le sommeil ou l'état de veille entre les patients dont l'ACV avait débuté pendant le sommeil et les contrôles. Cependant, les plaquettes étaient significativement plus sensibles à l'AA pendant le sommeil que pendant l'état de veille chez les patients dont l'ACV avait débuté pendant le sommeil (p <0.01). Cette différence se retrouvait seulement dans le sous-groupe de patients dont l'ACV avait débuté pendant le sommeil nocturne et non pendant le sommeil diurne, suggérant la possibilité que la différence observé dans la sensibilité plaquettaire à l'AA puisse être reliée à une fluctuation circadienne de l'agrégation plaquettaire plutôt qu'à une fluctuation reliée au sommeil. Aucun changement significatif dans l'agrégation plaquettaire n'a été identifié dans les deux autres groupes.

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If stroke occurs randomly, irrespective of sleep period or time of day, then, if the average individual spends eight hours a day sleeping, the incidence of sleep-related stroke onset should approximate 30%. This predicted incidence is closely approximated by an actual incidence of 34% reported by Mohr et al.[1] Caplan et al[2] found a 44% incidence of sleep-onset thrombotic stroke compared to a 31% incidence for embolic stroke, which suggested that thrombotic stroke occurs more commonly in relation to sleep than would be predicted by a random-onset hypothesis. Other authors have commented on the high incidence of thrombotic stroke onset during or immediately following sleep periods.[3] Adams and Victor write:[4]

"Even more frequent than the modes of onset outlined above, is the occurrence of thrombotic stroke during sleep. The patient awakens paralyzed, either during the night or in the morning . . . This is the story in fully 60% of our patients with thrombotic strokes . . . "

Pathological and clinical studies have indicated that platelets may be involved in the pathogenesis of cerebral ischemia[5-17] and clinical trials using anti-platelet drugs in patients with symptomatic cerebral ischemia tend to support the contention that platelet activation plays a role in stroke onset.[18,19]

We therefore decided to investigate platelet aggregability during sleep and wakefulness in patients with an established

predisposition to sleep-related thrombotic stroke. We hypothesized that, in a subgroup of the population at risk for thrombotic stroke, platelet activation occurs during sleep, and that this might predispose this subgroup to sleep-related stroke onset.

MATERIALS AND METHODS

Three patient groups, each comprising nine patients, were delineated:

(1) **Sleep-onset stroke (SOS) group:** Patients admitted with a history of stroke onset during sleep. Three of the nine patients in this group suffered stroke onset during the course of mid-afternoon naps.

(2) **Awake-onset stroke (AOS) group:** Age and sex-matched patients with a history of stroke onset whilst awake.

(3) **Control group (CONT):** Similarly matched patients with non-vascular neurologic or psychiatric disease. Only individuals without identifiable risk factors for vascular disease were included in this group.

Patients were selected from those seen in the Medical and Neurological Services of the Johannesburg Hospital. All patients were evaluated clinically by one of the authors (CLV) within 24 hours of presentation and were re-examined at one and seven days. Only patients with neurological deficit relating to the event and persisting for more than 24 hours were included. Evaluation included general and neurological examination, routine biochemical and hematological investigation, electrocardiogram, chest X-ray and computerized tomography (CT) of the brain to eliminate intracranial hemorrhage or non-ischemic lesions. Patients with CT evidence of recent non-hemorrhagic cerebral infarction or patients with normal CT scans but with clinical features of atherothrombotic infarction were included in the study. Patients with risk factors for cardioembolic stroke (atrial fibrillation, recent myocardial infarction, valvular heart disease, endocarditis) were not included. Informed consent was obtained from all subjects. In view of the possible risk involved in stopping prescribed anti-platelet drugs for ten days preceding evaluation of platelet aggregation, it was decided in conjunction with the Ethics Committee of the hospital that only patients without Doppler evidence of hemodynamically significant (> 50% carotid stenosis) extracranial vascular disease would be included.

Ten days before the study, participating patients were instructed to stop taking anti-platelet medications and other drugs that might affect platelet aggregation (acetylsalicylic acid, dipyridamole, sulfinpyrazone, non-steroidal anti-inflammatory agents, tricyclic antidepressants, estrogens, corticosteroids). They were instructed not to smoke or drink alcohol for 24 hours before the study. Determination of platelet aggregability was performed a minimum of seven weeks after initial presentation. A minimum seven week interval was chosen since the non-specific increase in platelet aggregability which occurs during the first ten days following stroke has been shown to resolve by six weeks.[13] Participants were admitted to the Neurological Unit on the afternoon of the study. That evening, EEG (C3-A2, C4-A1), EOG (1 cm above and below the outer canthus of each eye, referenced to A1) and EMG (mentalis) electrodes were affixed and calibrated. Lights were turned out at 23h00. Time of sleep onset was recorded and sleep phases were monitored utilizing standardized techniques.[20]

Three to six hours after sleep onset, a tourniquet was applied to the sleeping patient's arm and blood samples were obtained following tourniquet release from a fresh venipuncture site in an antecubital vein using an 18 gauge needle. An indwelling line was not used for blood sampling because of the possibility that thrombus formation at the tip of the venous catheter might cause platelet activation. Nine volumes of blood were drawn directly into 10 ml plastic syringes containing one volume of 3.2% trisodium citrate. A total of 50 ml of whole blood was sampled in this way for each series of aggregation studies. The samples were then transferred into polypropylene centrifuge tubes and inverted gently three times to ensure mixing of blood and anticoagulant. Between 11-13h00 on the following day, the patients having been awakened between 06-07h00, blood samples were taken from a fresh venipuncture site in the contralateral antecubital fossa. All platelet function tests were commenced within 30 minutes of sampling and were completed within 180 minutes of sampling.

Anticoagulated whole blood was centrifuged at 150 g for 10 minutes. The supernatant platelet-rich plasma (PRP) was separated into plastic tubes using a plastic-tipped automatic pipette. The remaining blood samples were centrifuged for 15 minutes at 1800 g and the supernatant was separated as before to obtain platelet-poor plasma (PPP). Platelet counts were determined using a thrombocounter (Coulter Electronics, Florida). For aggregation studies, the PRP was corrected to a platelet count of $250 \pm 10 \times 10^9/1$ utilizing autologous PPP.

Cuvettes used for the aggregation assays (7.9 mm diameter) were siliconized by immersion in 10% dimethyldichlorosilane (BDH Chemicals Ltd., Poole, England) /90% carbon tetrachloride (Merck Chemicals, Montreal) solution for 20 minutes. Platelet aggregation was measured in response to each of four agonists, namely ADP, epinephrine, collagen and AA (all reagents supplied by Sigma Chemical Company, St. Louis, USA) using the method of Born and Cross.[21] Aggregation assays were performed in a Chronolog dual channel aggregometer connected to an omniscribe recorder. For assays employing ADP and epinephrine as aggregants, 0.05 ml of agonist was added to 0.45 ml of platelet corrected plasma (PCP) in a prepared cuvette. Fresh serially graded solutions of ADP (0.03-5µg/ml), and epinephrine (0.03-10µg/m1) were prepared prior to each series of aggregation assays (see Appendix). For assays utilizing collagen and AA, 1-20 µl of agonist were added to 0.05 ml of PCP. Concentrations of collagen and arachidonic acid used were 0.06-4 µg/ml and 0.02-1.0 mM respectively. PCP and agonist were maintained at 37°C throughout the procedure and were stirred at 1000 rpm using a teflon coated magnetic stir bar. Aggregation was allowed to proceed for five minutes after the introduction of the stir bar to the cuvette. The lowest concentration of agonist that produced maximal aggregation, the threshold concentration, was determined according to the method of Chetty and Bradlow.[22] Maximal response is defined as the maximal change in optical density, five minutes from the introduction of the stir bar to the PCP/agonist solution. On completion of each series of aggregation studies, platelet aggregation response was checked at the determined threshold concentrations for each agonist.

Group mean threshold concentrations during sleeping and waking periods for each group were compared within each group and between groups using Student's T test for matched and independent variables respectively.

RESULTS

Clinical data are shown in Table 1. The group mean ages ± SEM were: 60.2 ± 5.3 (SOS), 65.6 ± 2.8 (AOS), 55.7 ± 4.1 (CONT). One SOS patient (1.4) gave a history of classical migraine since his teens; he had not experienced migraine symptoms during the day preceding stroke onset. One SOS patient (1.7) had been using a low-dose combined estrogen/progesterone oral contraceptive agent at the time of stroke onset. This had been discontinued following presentation. Three SOS patients (1.3, 7, 8) and two AOS patients (2.2, 4) had experienced one or more transient ischemic attacks involving the appropriate vascular territory, prior to stroke onset; all but two (1.3, 2.2) had been prescribed anti-platelet agents (acetylsalicylic acid ± dipyridamole) prior to stroke onset. Three patients in the SOS group (1.1, 4, 8) first noticed neurological deficit on awakening from mid-afternoon naps of approximately two hours duration. One patient (2.1) in the AOS group first became aware of neurological deficit just after midnight. He had not slept previously that evening. Two patients (1.8, 2.5) had undergone carotid endarterectomy, two and six years previously, respectively. Carotid Doppler evaluation revealed no hemodynamically significant residual disease in these patients. All patients in both stroke groups had neurological deficit relating to the left or right middle cerebral artery territory. Three SOS patients (1.1, 4, 6) and one AOS patient (2.1) had normal scans one week after the acute event.

Time of sleep onset, sleep duration before sampling and sleep phase at the time of sampling are shown (Table 2). There were no significant differences between groups in the mean sleep duration before sampling. No correlation was found between sleep stage immediately before sampling and platelet aggregability, or between platelet aggregability and the time elapsed from stroke until evaluation of platelet aggregability. The mean duration between stroke onset and evaluation of platelet aggregation was 45.0 ± 17.8 weeks (range: 7-171) for the SOS group and 30.6 ± 8.1 weeks (range: 7-77) for the AOS group.

Group Differences in Platelet Aggregability Using ADP, Epinephrine, Collagen and Arachidonic Acid

Group mean threshold concentrations during sleep and awake periods were calculated for each agonist:

ADP (Figure 1a, A): Platelet aggregability to ADP during both the awake and sleep phases was significantly greater in the AOS group than in the CONT group ($p<0.05$). No significant sleep-awake/day-night fluctuation in aggregability to ADP was found in any group.

Epinephrine (Figure 1a, B): No significant differences in threshold concentrations were found between the three groups or between sleep and awake periods within each group, when epinephrine was used as the agonist.

Collagen (Figure 1b, C): Significantly increased platelet responsiveness to collagen, both during sleeping and waking periods, was found in the AOS vs the SOS group ($p<0.01$).

Arachidonic acid (Figure 1b, D): Increased platelet responsiveness to AA was found in the AOS group, both during the sleep and waking periods, when compared to the SOS and CONT groups ($p<0.01$).

Table 1: Clinical Data

#	Age	Sex	Recovery Interval (weeks)	Stroke Onset (time)	Period Sleep/ Awake	Risk Factors	Exam	CT
				SOS				
1.1	87	F	7	15 00	s	ht,chol	Rm,d	n
1.2	76	M	11	00 00	s	sk	Rm,s,d	Lfp
1.3	73	M	85	08 00	s	sk,TIA	Rm,d	n
1.4	44	M	36	14 30	s	migraine	d	Lfp
1.5	59	M	16	06 00	s	ht,sk,oh	d	Lsc
1.6	48	F	7	05 30	s	ht,sk	Rm,s	n
1.7	44	F	24	07 00	s	sk,TIA,oc	Rm,d	Lp
1.8	45	M	171	16 00	s	sk,TIA,en	Lm	Rfp
1.9	66	M	48	05 00	s	sk,ht,fh	Rm,s	Lfp
				AOS				
2.1	55	M	11	00 15	w	ht,sk	Rm	n
2.2	76	M	77	15 30	w	ht,fh,TIA	Rm,d	Lp
2.3	70	M	37	21 30	w	sk,fh,oh	Lm,s	Rsc
2.4	67	F	40	22 00	w	ocad,TIA	Rm,s,d,v	Lfp
2.5	72	M	8	19 00	w	ht,sk,en	Rm,d	Lfp
2.6	75	M	14	08 00	w	ht,oh	Rm,d	Lfp
2.7	64	F	7	14 00	w	ht,sk,chl	Lm,s,v	Rp
2.8	59	F	35	12 00	w	ocad,chl	Rm,d	Lfp
2.9	53	M	46	21 30	w	ht,fh	Lm	Lsc
				CONT				
						clinical diagnosis		
3.1	68	F				cervical spondylosis		
3.2	59	M				multiple sclerosis		
3.3	45	F				multiple sclerosis		
3.4	41	F				multiple sclerosis		
3.5	72	M				motor neuron disease		
3.6	49	M				depression		
3.7	56	M				multiple sclerosis		
3.8	42	M				motor neuron disease		
3.9	68	M				post traumatic epilepsy		

#:	Number allocated to patients in study. First integer indicates group. Second indicates patient number in that group.
sleep/awake:	activity at stroke onset
M/F:	male/female
ht:	hypertension
chl:	hypercholesterolemia (high normal range for age 33)
sk:	smoking
oc:	combined oral contraceptive (current/recent past usage)
en:	carotid endarterectomy
fh:	family history stroke/myocardial infarction
oh:	alcohol abuse
ocad:	symptomatic occlusive coronary artery disease or ECG features thereof.
R/L:	right/left
m:	hemiparesis, hemiplegia
s:	hemihypesthesia
d:	dysphasia, aphasia
v:	homonymous hemianopia
n:	normal
fp:	frontoparietal infarct
sc:	subcortical infarct
p:	parietal infarct
SOS:	sleep onset stroke group
AOS:	awake onset stroke group
CONT:	control group

Sleep-Awake (Day-Night) Fluctuations in Platelet Aggregation

Platelet aggregation to AA was significantly increased during sleeping vs waking periods in the SOS group only (p<0.01). Eight of the nine patients in this group had increased platelet aggregability to AA during the night following 3-6 hours of sleep. In contrast only two patients in the AOS group had increased platelet aggregability to AA during the night. One of the two (2.1) had first become aware of stroke onset at 00ʰ15 having not slept previously that night. Five AOS patients had

Table 2: Sleep Monitoring

#	Sleep Onset (Clock Time)	Sleep Duration (Minutes)	Sleep Phase (Before Sampling)
	SOS		
1.1	23 00	180	N REM 1
1.2	21 00	180	NA
1.3	22 15	225	N REM 4
1.4	23 30	240	N REM 2
1.5	21 10	200	N REM 2
1.6	20 00	360	REM
1.7	19 45	325	REM
1.8	00 00	240	N REM 2
1.9	21 00	210	REM
		240.0 ± 20.9*	
	AOS		
2.1	19 30	285	N REM 2
2.2	22 30	240	N REM 2
2.3	20 53	232	N REM 3
2.4	20 15	225	N REM 3
2.5	22 00	360	NA
2.6	22 00	235	REM
2.7	21 00	240	NA
2.8	19 45	225	NA
2.9	20 00	225	REM
		251.8 ± 14.9*	
	CONT		
3.1	20 43	217	N REM 2
3.2	20 15	260	REM
3.3	22 05	130	N REM 2
3.4	22 00	180	N REM 2
3.5	19 15	287	N REM 2
3.6	20 28	297	REM
3.7	22 30	180	NA
3.8	23 30	180	REM
3.9	19 30	180	N REM 3
		212.3 ± 19.0*	

* mean ± SEM
#1: Number allocated to patients in study. First integer indicates group. Second indicates patient number in that group.
sleep duration: sleep duration as calculated from time of sleep onset to time of waking during blood sampling.
sleep phase: NREM 1-4: Non-REM or slow wave sleep, stages 1-4
REM: Rapid eye movement sleep
NA: Sleep phase not known because of technical difficulties with recording (ie, electrode displacement after sleep onset — in these cases subjects were carefully observed to determine sleep/awake periods).
SOS: sleep onset stroke group
AOS: awake onset stroke group
CONT: control group

relatively increased platelet responsiveness to AA during the day. Two showed no change. In the CONT group, seven of nine patients showed no change in response to AA between day vs night samples. One had relatively increased platelet aggregability to AA during the night and one had increased daytime aggregability.

Platelet Aggregation in Day vs Night Sleep-Onset Stroke

Three patients in the SOS group first became aware of neurological deficit on awakening from afternoon naps. A separate analysis of data from these three patients was performed since it was not possible to assume a priori that stroke onset during daytime vs nocturnal sleep were equivalent. Mean threshold concentrations for the four agonists during sleep vs awake periods in patients who had suffered stroke onset during the course of afternoon naps were not significantly different. However, in patients who had suffered stroke onset in relation to nocturnal sleep, a significantly reduced threshold concentration during the sleep vs the awake sampling period was found for AA but not for the other agonists (T = 5.0, p = 0.004).

DISCUSSION

Our results showed that platelets from the group which had suffered stroke onset whilst awake were significantly more aggregable than were those from the control and sleep onset stroke groups. This might be a reflection of the increased mean age in the AOS vs the other two groups since platelet function has been shown to increase as a function of age.[15] However the differences in age between the three groups were not found to be significant. Alternatively, this may be a reflection of the higher incidence of hypertension in the AOS group, since there is a direct correlation between hypertension and increased platelet aggregability.[23] The second finding to emerge from this study was that platelet responsiveness to AA was significantly greater during the night than during the day in patients who first became aware of neurological deficit following awakening from a period of nocturnal sleep. In contrast, no significant differences in aggregability to AA were found between night and daytime samples in the AOS and CONT groups. Indeed, the large number of assays which showed no change in aggregation threshold between sleep and awake periods confirmed a high degree of consistency in the determination of aggregation thresholds.

It was not established from this study whether the change in platelet responsiveness to AA found in the SOS patients between sleeping and waking periods is related to the sleep-wake cycle or to an underlying circadian fluctuation in platelet aggregability. Previous studies have suggested that platelet aggregability is subject to circadian fluctuation.[24,25] When SOS patients were subdivided into day vs nighttime sleep-related stroke onset, the sleep-awake fluctuation in sensitivity to AA was confined to the night-sleep onset subgroup suggesting perhaps that the observed difference in responsiveness to AA between sleep and awake samples was a reflection of an underlying diurnal variation in platelet aggregability, rather than of a sleep phase-related phenomenon. Several studies have previously reported increased platelet aggregability to AA in cerebrovascular disease.[26,27] Why sleep-awake fluctuation in platelet aggregability was confined to assays employing AA is uncer-

tain. Platelet AA metabolism is subject to modulation by circulating catecholamines.[28] Sleep-associated increases in circulating catecholamines[29] might account for this observation. Individual variation in sleep-associated increases in circulating catecholamines, or in the modulatory effect of catecholamines on platelet cyclo-oxygenase could explain why this finding was confined to one subgroup of the study population. Circadian fluctuations of AA metabolism[30] might modulate platelet function in-vivo.[28]

Four of the patients in the control group had multiple sclerosis. There is evidence that platelets may be hyperaggregable during or shortly after acute exacerbations of the disease.[31,32] However, none of the patients with multiple sclerosis included in this study had experienced recent acute exacerbations. It is

unlikely therefore that this factor affected the results and if so, it would be expected to have reduced differences in platelet aggregability between control and stroke groups.

Although our findings do not suggest a causal relationship between circadian or sleep-related fluctuations in platelet aggregation and time of stroke onset, they do demonstrate significant differences in platelet responsiveness in association with awake vs sleep-onset stroke and suggest that platelet aggregability varies in association with the time of stroke onset. Little is currently known of the mechanisms initiating thrombotic stroke onset. Clarification of these mechanisms would be of benefit in furthering the understanding of stroke in general and in helping to identify different sub-populations at risk for stroke during differing activities of daily living.
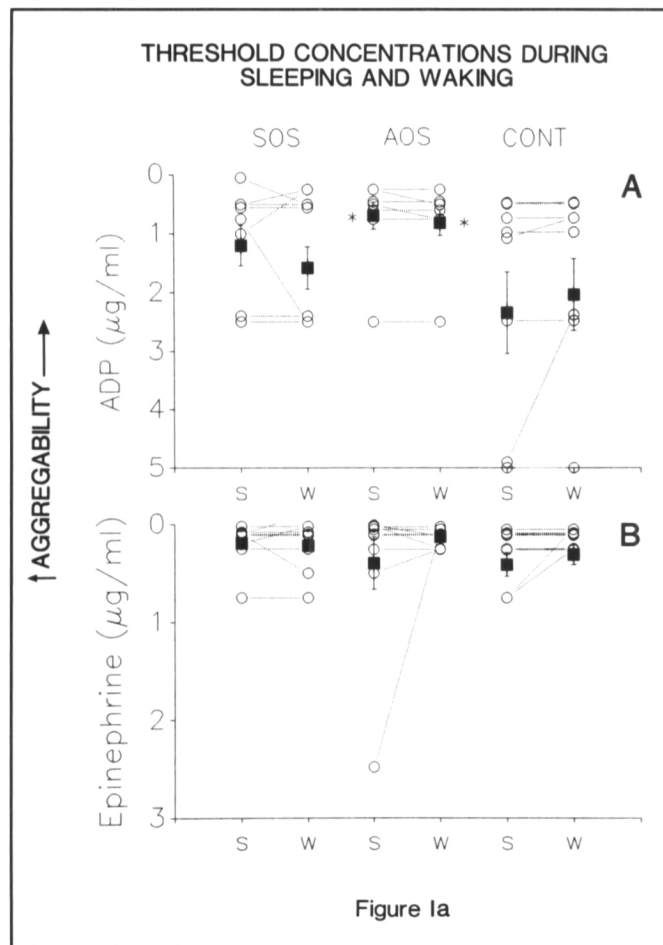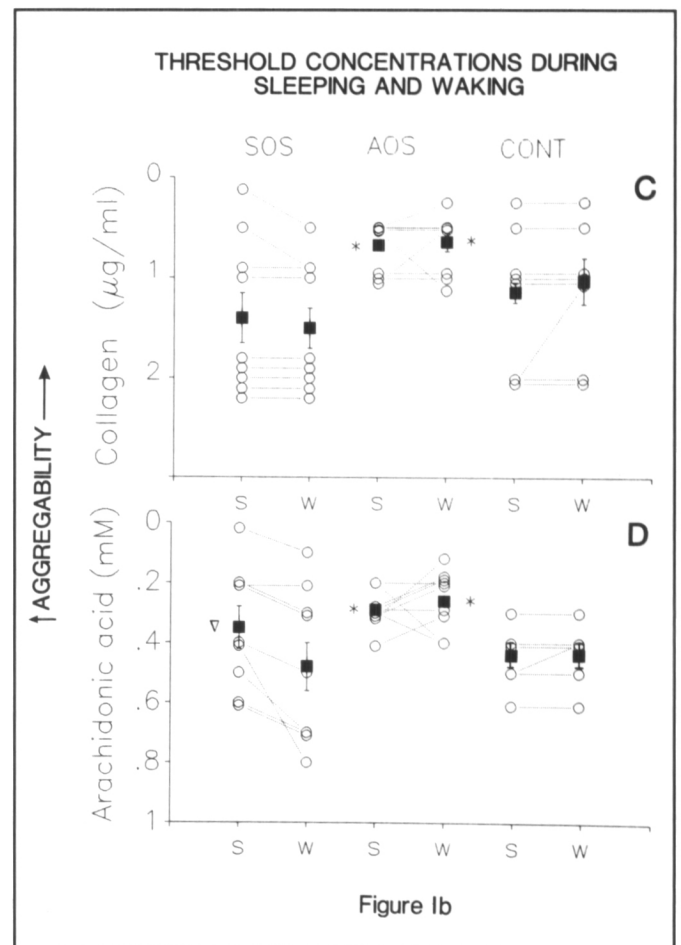

Figure Ia

Figure Ib

Figure 1a and b — Open circles: individual aggregation threshold values. Filled squares and error bars: group mean ± SEM.
  (A) ADP: * significantly < cont group (asleep and awake), p<0.05, T Test.
  (B) Epinephrine: No significant intra- or inter-group differences.
  (C) Collagen: * significantly < SOS group (asleep and awake), p<0.01, T Test.
  (D) Arachidonic acid: ∇ significantly < SOS group (awake),
                p<0.01, T Test.
                * significantly < cont group (asleep and
                awake) and SOS group (awake), p<0.01,
                T Test.
  SOS   Sleep onset stroke group
  AOS   Awake onset stroke group
  CONT Control group
  S   Sleeping phase
  W   Waking phase

APPENDIX: Agonist Concentrations Employed:

ADP (µg/ml):	5	2.5	1	0.75	0.5	0.1	0.05	0.03			
EPINEPHRINE (µg/ml):	10	5	2.5	1	0.75	0.5	0.1	0.05	0.03		
COLLAGEN (µg/ml):	4	2	1	0.5	0.25	0.12	0.06				
ARACHIDONIC ACID (mM):	1	.9	.8	.7	.6	.5	.4	.3	.2	.1	.05 0.02

Individual Aggregation Thresholds During Sleeping and Waking

	ADPµg Sleep	ADPµg Awake	EPIµg Sleep	EPIµg Awake	COLLµg Sleep	COLLµg Awake	AAmM Sleep	AAmM Awake
				SOS				
1.1	2.5	2.5	0.75	0.75	2	2	0.6	0.7
1.2	0.05	0.5	0.02	0.02	0.12	0.5	0.02	0.1
1.3	0.5	0.5	0.2	0.02	1	1	0.2	0.3
1.4	0.5	0.5	0.1	0.1	2	2	0.5	0.7
1.5	0.75	2.5	0.1	0.1	1	1	0.2	0.2
1.6	2.5	2.5	0.1	0.1	2	2	0.6	0.7
1.7	2.5	2.5	0.25	0.25	2	2	0.4	0.5
1.8	0.5	0.25	0.1	0.5	2	2	0.4	0.8
1.9	1	2.5	0.1	0.1	0.5	1	0.2	0.3
				AOS				
2.1	0.75	0.75	0.1	0.1	0.5	0.5	0.3	0.4
2.2	0.5	0.75	0.05	0.05	1	1	0.3	0.2
2.3	0.25	0.2	0.03	0.03	0.5	0.25	0.3	0.1
2.4	2.5	2.5	2.5	0.0	1	1	0.4	0.3
2.5	0.5	0.75	0.1	0.1	1	0.5	0.3	0.2
2.6	0.25	0.5	0.02	0.25	0.5	0.5	0.2	0.2
2.7	0.5	0.5	0.01	0.12	0.5	1	0.2	0.4
2.8	0.5	0.5	0.25	0.25	0.5	0.5	0.3	0.2
2.9	0.5	0.75	0.5	0.25	0.5	0.5	0.3	0.3
				CONT				
3.1	0.5	0.5	0.05	0.05	0.25	0.25	0.3	0.3
3.2	0.5	0.5	0.1	0.1	0.5	0.5	0.4	0.4
3.3	1	1	0.75	0.5	1	1	0.5	0.5
3.4	1	0.75	0.25	0.25	2	2	0.5	0.5
3.5	5	5	1	1	1	1	0.4	0.4
3.6	2.5	2.5	0.75	0.25	2	1	0.6	0.6
3.7	5	5	0.1	0.1	1	1	0.5	0.4
3.8	5	2.5			2	2	0.3	0.4
3.9	0.75	0.75	0.25	0.25	0.5	0.5	0.3	0.3

SOS: sleep onset stroke group
AOS: awake onset stroke group
CONT: control group

REFERENCES

1. Mohr JP, Caplan LR, Melski JW, et al. The Harvard co-operative stroke registry: A prospective registry. Neurology 1978; 28: 754-762.
2. Caplan LR, Hier DB, D'Cruz I. Cerebral embolism in the Micheal Reese stroke registry. Stroke 1983; 14: 530-536.
3. Carter AB. Clinical aspects of cerebral infarction. In: Vinken PJ, Bruyn GW, eds. Vascular Diseases of the Nervous System-Part I, Handbook of Clinical Neurology. Amsterdam: North Holland Publishing Company and New York: American Elsevier Publishing Co 1972; 292-326.
4. Adams RD, Victor M. Cerebrovascular diseases. In: Barry BK, Schwarz M, Scott EJ, eds. Principles of Neurology. New York: McGraw Hill Book Company 1985; 569-640.
5. Meyer JS. Summary of the International Round Table Conference on platelet aggregation in the pathogenesis of cerebrovascular disorders. Stroke 1975; 6: 239-244.

6. Harker LA, Ritchie JI. The role of platelets in acute vascular events. Circulation (Suppl 5) 1980; 13-17.
7. Fleischman AI, Bierenbaum ML, Justice D, et al. In vivo platelet function in acute myocardial infarction, acute cerebrovascular accidents and following surgery. Thromb Res 1975; 6: 205-207.
8. Harker LA, Slichter J. Arterial and venous thromboembolism: Kinetic characterization and evaluation of therapy. Throm Diath Haemorrh 1974; 31: 188-203.
9. Uchiyama S, Takeuchi M, Osawa M, et al. Platelet function tests in thrombotic cerebrovascular disorders. Stroke 1984; 14: 511-517.
10. Cate JW, Vos J, Oosterhuis H, et al. Spontaneous platelet aggregation in cerebrovascular disease. Stroke 1978; 39: 223-229.
11. Wu KK, Hoak JC. Increased platelet aggregates in patients with transient ischemic attacks. Stroke 1975; 6: 521-524.
12. Sano T, Boxer MGJ, Boxer LA, et al. Platelet sensitivity to aggregation in normal and diseased groups: A method for assessment of platelet aggregability. Thromb Diath Haemorrh 1971; 25: 524-531.
13. Dougherty JH, Levy DE, Weksler BB. Platelet activation in acute cerebral ischemia. Serial measurements of platelet function in cerebrovascular disease. Lancet 1977; 1: 821-824.
14. Danta G. Second phase platelet aggregation by adenosine diphosphate in patients with cerebral vascular disease and in normal

subjects. Thromb Diath Haemorrh 1979; 23: 159-169.

15. Couch JR, Hassenein RS. Platelet aggregation, stroke, and transient ischemic attack in middle-aged and elderly patients. Neurology 1976; 26: 888-895.

16. Kalendovsky Z, Austin J, Steele P. Increased platelet aggregability in young patients with stroke. Arch Neurol 1975; 32: 13-29.

17. Otsuki Y, Kondo T, Shio H, et al. Platelet aggregability in cerebral thrombosis analyzed for vessel stenosis. Stroke 1983; 14: 368-371.

18. Fields WS, Lemak NA, Frankowski RF, et al. Controlled trial of aspirin in cerebral ischemia. Stroke 1977; 8: 301-316.

19. Bousser MG, Escwege E, Haguenau M, et al. "AICLA" controlled trial of aspirin and dipyridamole in the secondary prevention of atherothrombotic cerebral infarction. Stroke 1983; 14: 5-14.

20. Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington: US Government Printing Office, 1968.

21. Born GVR, Cross MJ. The aggregation of blood platelets. J Physiol 1963; 168: 179-195.

22. Chetty N, Bradlow BA. The effects of a vegetarian diet on platelet function and fatty acids. Thromb Res 1983; 30: 619-624.

23. Cocherri S, Fiorentini P. Platelet adhesiveness and aggregation in hypertensive patients. Acta Med Scand 1971; 525: 273-275.

24. Nubile G, D'Alonzo L, Consoli A, et al. Variazione circadiana dell aggregazione piastrinica indotta da adrenalina e collagene nell'uomo. Boll Soc Ital Biol Sper 1982; 58: 947-950.

25. Tofler GH, Brezinski D, Shafer AI, et al. Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. N Eng J Med 1987; 316: 1514-1518.

26. Sié P, Perret B, Cousin F, et al. Platelet arachidonic acid metabolism in severe cerebrovascular disease. Thromb Res 1982; 28: 1-9.

27. Matsumoto M, Nukada T, Uyama O, et al. Thromboxane generation in patients with essential hypertension or cerebrovascular disease and effect of oral aspirin. Thromb Haemost 1980; 44: 16-22.

28. Rao GHR, White JG. Role of arachidonic acid metabolism in human platelet activation and irreversible aggregation. Am J Hematol 1985; 19: 339-347.

29. Parker DC, Rossman LG, Kripke DF, et al. Endocrine rhythms across sleep-wake cycles in normal young men under basal conditions. In: Orem J, Barnes CD, eds. Physiology in sleep. Academic Press Inc, New York 1980; 145-179.

30. Rigas B, Levine L. Human salivary eicosanoids: Circadian variation. Biochem Biophys Res Commun 1983; 115: 201-205.

31. Couch JR, Hassenein RS. Platelet hyperaggregability in multiple sclerosis. Trans Am Neurol Assoc 1977; 102: 62-64.

32. Neu I, Prosiegel M, Pfaffenrath V. Platelet aggregation and multiple sclerosis. Acta Neurol Scand 1982; 66: 497-504.

33. Fredrickson DS, Levy RI, Lees RS. Fat transport in lipoproteins — an integrated approach to mechanisms and disorders. N Eng J Med 1967; 276: 149-156.