Calcium Independent Contraction Induced by Iodoacetic Acid in Isolated Cerebral Arteries

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SUMMARY In helically-cut strips of cerebral arteries isolated from dogs and monkeys, the addition of 1 mM iodoacetic acid (IAA) produced contractions during an early period (5 to 10 min) and also a prolonged exposure (50 to 70 min). The early contraction was abolished by exposure to Ca^{++}-free media containing EGTA, and significantly attenuated by treatment with procaine or dantrolene. Verapamil, lidocaine, ATP and pyruvate did not inhibit the contraction. On the other hand, the late contraction was not prevented by exposure to Ca^{++}-free, EGTA-containing media and by treatment with procaine, dantrolene, lidocaine, ATP or pyruvate. Nitroglycerin and papaverine did not relax the IAA-contracted arteries. In dog and monkey mesenteric arteries and dog coronary, renal and femoral arteries, IAA elicited contractions after a prolonged exposure, which were not inhibited by soaking the preparations in Ca^{++}-free, EGTA-containing media. Passive tensions developed by rapid stretch in Ca^{++}-free media did not differ in IAA-treated and control arteries. During an early period of IAA actions, Ca^{++} appears to be released from intracellularly stored sites in the amount sufficient to produce significant contractions in cerebral, but not in peripheral arteries. It is concluded that the involvement of Ca^{++} in the late contraction induced by IAA is if any minimal, and such a contraction may be associated with functional alterations induced by the metabolic inhibitor in arterial tissues other than smooth muscle.

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CEREBRAL VASOSPASM following subarachnoid hemorrhage is postulated to result from exposure to vasoconstrictor substances, such as serotonin,[1,2] K^+,[3] hemoglobin,[4] and thrombin[5] originated from blood surrounding cerebral vessels, and prostaglandins[6-9] produced secondarily by blood constituents. However, the spasm usually arises after several days of exposure to blood clot and hemolysate and is quite resistant to treatment with vasodilators, which are expected to reduce the amount of active Ca^{++} in the vicinity of contractile proteins. Prolonged impairment of metabolism in cerebrovascular muscle cells, possibly by circulatory disturbance of vasa vasorum, may bring the vessels into the rigor state, under which contractions independent of Ca^{++} can be observed.[10]

Iodoacetic acid (IAA) interferes with anaerobic glucose metabolism and depletes the content of ATP in cardiac and smooth muscles.[11-14] IAA-induced rigor appears to be associated with such a depletion of cellular ATP.[15,16] However, Lundholm and Mohme-Lundholm[12] failed to see a rigor-like contraction in isolated bovine mesenteric arteries treated with 22 mM IAA, despite a marked depletion of ATP.

The purpose of the present study was to quantitatively compare the response to IAA of cerebral and peripheral arteries isolated from dogs or monkeys, and to analyze the mechanism underlying the contraction of isolated cerebral arteries during short and prolonged periods of exposure to IAA.

Methods

Mongrel dogs of either sex, weighing 8 to 16 kg, were anesthetized with intraperitoneal injections of sodium pentobarbital (50 mg/kg) and killed by bleeding from the common carotid arteries. The brain, the heart and kidneys were rapidly removed. Basilar and middle cerebral arteries (0.6 to 0.8 mm outside diameter) were isolated from the brain, interventricular and circumflex branches of the left coronary artery (0.7 to 0.9 mm) were isolated from the heart, and intrarenal, interlobar branches of the renal artery (0.6 to 0.8 mm) were isolated from the kidney. Distal portions of the superior mesenteric and femoral arteries (0.6 to 0.9 mm) were also isolated. Japanese monkeys (Macaca fuscata) of either sex, weighing 7 to 10 kg, were anesthetized with intramuscular injections of ketamine (60 mg/kg) and killed by bleeding from the carotid arteries. Basilar, middle cerebral (0.6 to 0.8 mm) and superior mesenteric arteries (0.5 to 0.7 mm) were isolated. The arteries were helically cut into strips approximately 20 mm long. The specimen was vertically fixed between hooks in a muscle bath (20 ml capacity) containing the nutrient solution. Constituents of the solution were as follows (mM): NaCl 120, KCl 5.4, $CaCl_2$ 2.2, $MgCl_2$ 1.0, $NaHCO_3$ 25.0, and dextrose 5.6. The solution was maintained at 37 ± 0.3°C and aerated with a mixture of 95% O_2 and 5% CO_2. In some preparations, the solution was aerated with 95% N_2 and 5% CO_2. The pH of the solution was 7.3 to 7.4. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihonkohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g for dog arteries and to 1.0 g for monkey arteries. Before the start of experiments, the strips were allowed to equilibrate for 60 to 90 min in the bathing media. During the equilibration period, the media were replaced every 10 to 15 min.

Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Nihonkohden Kogyo Co.). The contractile response to 30 mM K^+ was first obtained, then the preparations were washed three times with control media and allowed to equilibrate for 40 to 50 min. IAA was added directly to the bathing media. Contractions induced by IAA relative

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to those induced by 30 mM K^+ were presented. Preparations had been treated for 30 min with blocking agents or pyruvate before IAA was added. In strips treated with ATP, IAA was added after the transient contraction induced by ATP was abolished and the tension was stabilized. Hemolysate was prepared with blood collected from the carotid artery of dogs, following procedures described in the previous report.[8] The hemoglobin content was measured as a standard of concentrations of the hemolysate. Some preparations were exposed to Ca^{++}-free media containing EGTA for 60 min during which time the media were replaced twice every 20 min, or for 180 min during which time the fluids were replaced twice every 60 min. In 4 mesenteric arterial strips equilibrated for 120 min in the bathing media, the response to 30 mM K^+ was obtained. The preparations were washed three times with control media and equilibrated for 40 min. Then, the strips were removed from the muscle bath and soaked for 24 hours in Ca^{++}-free, EGTA (1 mM)-containing nutrient solutions at 4°C. After the incubation period, these strips were again fixed between hooks in the muscle bath containing Ca^{++}-free, EGTA-added media at 37°C. After a 120 min equilibration period, IAA was added.

Arterial strips were stretched rapidly by 20% of the initial length (dog cerebral artery) or by 40% of the initial length (dog mesenteric artery). Time course of the passive tension developed was recorded. The initial tension rapidly attained and the tension stabilized during an observation period of 10 to 40 min were measured. Details of the experimental procedures were described in an earlier report.[17] The cross sectional area of the arterial strips was estimated by the ratio of wet weight to initial length.

Results shown in the text, tables and figures are expressed as mean values ± SEM. Statistical analyses were made using the Student's paired and unpaired t test. Drugs used were iodoacetic acid (IAA), ethylene glycol-bis-(β-aminoethylether)-N,N′-tetraacetic acid (EGTA), dantrolene sodium (Yamanouchi Pharmaceutical Co., Tokyo), procaine hydrochloride, lidocaine hydrochloride, dl-verapamil hydrochloride, adenosine triphosphate (ATP), pyruvic acid, nitroglycerin, sodium nitroprusside, papaverine hydrochloride and prostaglandin $F_{2\alpha}$.

Results

Effects of IAA on Different Arteries Isolated from Dogs and Monkeys

In helically cut strips of dog cerebral arteries, the addition of 1 mM IAA produced a transient contraction (25.0 ± 4.4%, n = 25, relative to contractions induced by 30 mM K^+), the time required to attain the maximum contraction being 7.7 ± 1.1 min. The tension returned to a level lower than that prior to the addition of IAA. A secondary contraction (12.5 ± 2.4%, n = 25) was observed after 50 to 70 min exposure to IAA; the time to the peak tension averaged 82.1 ± 2.4 min. The late contraction persisted for 25 to 50 min. Typical recordings of the response to IAA are shown in figure 1, upper tracing. Middle cerebral arteries tended to respond to 1 mM IAA with a greater contraction during the early period than basilar arteries (32.2 ± 6.4%, n = 16, vs. 14.7 ± 4.0%, n = 9); however, the difference was statistically insignificant. Late contractions of middle cerebral and basilar arteries did not differ (11.1 ± 1.2 and 15.1 ± 6.5%, respectively). Similar magnitude of the late contraction (18.5 ± 5.8%, n = 6, with the average time to peak contraction of 80.1 ± 4.5 min) was obtained in response to IAA in the bathing media aerated for 30 min with a mixture of 95% N_2 and 5% CO_2. In normal media, increase in the concentration of IAA to 5 mM produced a lesser magnitude of the early contraction (9.3 ± 2.8%, n = 5) and a greater magnitude of the late contraction (22.6 ± 1.3%, n = 5). The late contraction developed earlier; the time to attain the maximum contraction averaged 39.8 ± 3.6 min, which was significantly different from the value of 82.1 ± 2.4 min (n = 25) with 1 mM IAA ($p < 0.001$). The time to restore half the maximum contraction was significantly increased. The results are summarized in table 1. Once the late contraction was established, ATP (1 mM) or vasodilator agents, including papaverine (10^{-4} to 5×10^{-4} M), nitroglycerin (10^{-4} M), sodium nitroprusside (10^{-4} M) and verapamil (10^{-6} M), did not relax the arteries. Further, these arterial strips were unresponsive to 30 mM K^+ or 3×10^{-6} M prostaglandin $F_{2\alpha}$.

In contrast to dog cerebral arteries, helical strips of monkey cerebral arteries responded to 1 mM IAA with a marked, transient contraction (130 ± 13.9%, n = 9, relative to contractions induced by 30 mM K^+) followed by a late contraction (10.9 ± 2.5%). Average times to the peak of the early and late contractions were 9.0 ± 1.3 and 69.3 ± 3.6 min, respectively. Recordings of the response of a monkey basilar arterial strip are demonstrated in figure 2, upper tracing.

In helical strips of dog mesenteric, coronary, renal and femoral arteries, the addition of 1 mM IAA did not produce contractions during the early period but did elicit contractions after 60 to 90 min exposure. Average times to attain the peak contractions were 103 ± 8.5 (n = 13), 116 ± 14.8 (n = 11), 119 ± 5.9 (n = 14) and 107 ± 13.7 min (n = 8), respectively. The magnitude of the late contraction was greater in mesen-

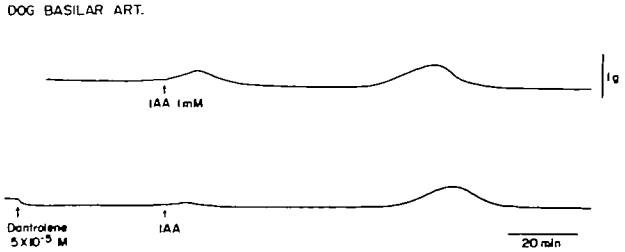

FIGURE 1. *Responses to 1 mM IAA of basilar arterial strips obtained from the same dog. Upper recording, control; lower, dantrolene-treatment. Contractions induced by 30 mM K^+ for upper and lower recordings were 2721 and 3105 mg, respectively.*

TABLE 1 *Effects of IAA on Helical Strips of Different Arteries Isolated from Dogs and Monkeys*

Artery	IAA (mM)	N	K^+ 30 mM contraction (mg)	IAA-induced Early contraction (%)*	IAA-induced Early contraction (mg)	IAA-induced Late contraction (%)*	IAA-induced Late contraction (mg)	IAA-induced Late contraction ½ D (min)	Tension reduction† (mg)
Dog									
cerebral	1	25	1789 ± 154	25.0 ± 4 4	363 ± 60	12.5 ± 2.4	170 ± 28	16.8 ± 0.9	409 ± 53
cerebral	5	5	1302 ± 329	9.3 ± 2.8	135 ± 63	22 6 ± 1 3	295 ± 75	26 0 ± 2.6‡	344 ± 68
mesenteric	1	13	2888 ± 310§	0‡	0‡	27.3 ± 6.1|	1097 ± 442	64.6 ± 10.1‡	41 ± 8‡
coronary	1	11	2274 ± 307	0‡	0‡	11.6 ± 1.0	394 ± 70	87.0 ± 3.1‡	188 ± 57|
femoral	1	8	3202 ± 372	0‡	0‡	20.4 ± 6.7	750 ± 237	51.4 ± 7.6‡	49 ± 16‡
renal	1	14	3028 ± 412	0‡	0‡	16.1 ± 3.0	693 ± 174	68.4 ± 7.5‡	38 ± 4‡
Monkey									
cerebral	1	9	1679 ± 339	130 ± 13.9‡	1963 ± 322‡	10.9 ± 2.5	190 ± 40	23.0 ± 6 4	347 ± 138
mesenteric	1	5	1284 ± 213	3.8 ± 1.9¶	35 ± 11¶	4 8 ± 1 8	67 ± 23	40.8 ± 5.9	45 ± 19

*Contractions relative to those induced by 30 mM K^+; †Decrease in the tension as compared with the level prior to the addition of IAA. N = number of preparations used; ½ D = duration of half the magnitude of late contractions.
‡Significantly different from the value in dog cerebral arteries, $p < 0.001$; §$p < 0.01$; | $p < 0.02$.
¶Significantly different from the value in monkey cerebral arteries, $p < 0.001$

teric arterial strips, but did not significantly differ in coronary, renal and femoral arteries, as compared with that seen in cerebral arteries (table 1). The contraction persisted longer in the peripheral arteries. Monkey mesenteric arterial strips when exposed to 1 mM IAA for 50 to 100 min responded with a contraction (table 1); the mean time to attain the peak contraction was 101 ± 16.8 min (n = 4).

Modification by Antagonists of the Response to IAA

Effects of various antagonists on the contractile response to 1 mM IAA of dog cerebral arteries are summarized in table 2. Treatment of the arterial strips for 60 or 180 min with Ca^{++}-free media containing 1 mM EGTA abolished the IAA-induced early contraction. However, the late contraction was rather potentiated and prolonged. Even when the concentration of EGTA in Ca^{++}-free media was increased to 5 mM and preparations were treated for 180 min, the late contraction was also induced (13.0 ± 1.7%, n = 3). Verapamil in a concentration of 10^{-6} M did not significantly alter the early and late contractions. Dantrolene (5×10^{-5} M) and procaine (5×10^{-3} M) significantly attenuated the magnitude of early contractions but did not influence the late contraction. Dantrolene relaxed the cerebral arteries (51 ± 12 mg, n = 11), while procaine moderately contracted the arteries (609 ± 54 mg, n = 8). In experiments with a pair of strips of cerebral arteries from the same dogs (n = 7), one as a control and the other for treatment with dantrolene, the early contraction was significantly attenuated by the treatment (28.9 ± 6.6 vs. 12.4 ± 2.9%, $p < 0.05$), whereas the late contraction was unaffected (11.3 ± 2.2 vs. 10.3 ± 2.0%) (fig. 1). Treatment with 5 mM lidocaine, 1 mM ATP and 10 mM pyruvate did not significantly alter the IAA-induced contractions. ATP rapidly contracted the arteries (706 ± 118 mg, n = 7), in which the tension returned to a level lower than that prior to the addition of ATP within 15 min. Pyruvate relaxed the arterial strips by 125 ± 43 mg (n = 5).

In cerebral arterial strips contracted for 30 min with 10^{-6} M prostaglandin $F_{2\alpha}$ (518 ± 74 mg, n = 6), IAA (1 mM) produced a transient contraction, as in the arteries under resting conditions, which was followed by a relaxation to a level prior to the addition of prostaglandin. In the arterial strips contracted with hemolysate (0.1 g/dl as a concentration of hemoglobin; 1190 ± 403 mg, n = 3), IAA-induced early contractions were increased (table 2). The tension returned to the initial level. The late contraction was not altered.

Exposure of monkey cerebral arteries to Ca^{++}-free solutions containing 1 mM EGTA for 180 min suppressed the early contraction induced by 1 mM IAA to 3.1 ± 1.7% (n = 5) from the average contraction of 130 ± 13.9% (n = 9) in control strips (fig. 2, lower tracing). The late contraction was not reduced by removal of external Ca^{++} (11.4 ± 1.4%, n = 5).

In mesenteric arterial strips exposed for 180 min or 24 hours to Ca^{++}-free, EGTA (1 mM)-containing media, IAA (1 mM)-induced contractions (34.0 ± 2.5%,

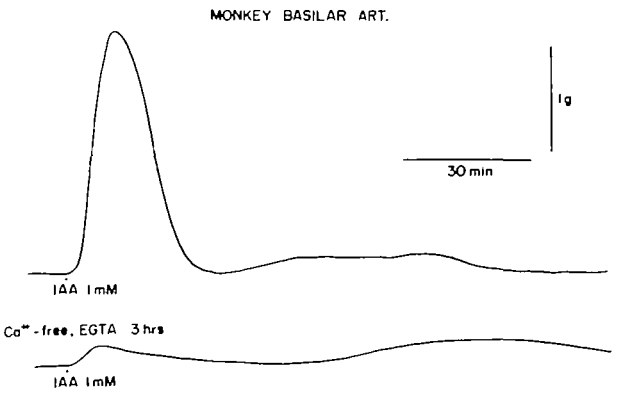

FIGURE 2. *Responses to 1 mM IAA of basilar arterial strips obtained from the same monkey. Upper recording, control; lower, exposure for 180 min to Ca^{++}-free media containing 1 mM EGTA. Contractions induced by 30 mM K^+ for upper and lower recordings were 2130 and 2306 mg, respectively.*

TABLE 2 *Modification by Antagonists of the Responses of Dog Cerebral Arteries to 1 mM IAA*

Treatment	N	K^+ 30 mM Contraction (mg)	IAA-induced					Tension reduction† (mg)
			Early contraction		Late contraction			
			(%)*	(mg)	(%)*	(mg)	½ D (min)	
None	25	1789 ± 154	25.0 ± 4.4	363 ± 60	12 5 ± 2.4	170 ± 28	16.8 ± 0.9	409 ± 53
Ca^{++}-free, EGTA 1 mM, 60 min	4	1345 ± 229	0‡	0‡	35 0 ± 7.3§	430 ± 33§	37.5 ± 7.5‡	90 ± 11¶
Ca^{++}-free, EGTA 1 mM, 180 min	7	1443 ± 363	0‡	0‡	27.3 ± 4.1§	355 ± 62§	40.0 ± 3.1‡	63 ± 30§
Verapamil 10^{-6} M	7	2106 ± 240	11.0 ± 4.0	173 ± 45	7.6 ± 1.3	117 ± 16	18.9 ± 2.2	132 ± 30|
Dantrolene 5 × 10^{-5} M	11	1754 ± 229	9.1 ± 2.2¶	143 ± 36¶	9.4 ± 1.4	176 ± 40	18.2 ± 2.5	244 ± 57
Procaine 5 × 10^{-3} M	8	1141 ± 99	4.4 ± 2.0|	53 ± 25|	13.6 ± 2.4	159 ± 28	22 4 ± 2.0	327 ± 85
Lidocaine 5 × 10^{-3} M	5	1396 ± 158	12.8 ± 4.5	172 ± 70	11 0 ± 1.6	154 ± 30	15.0 ± 2.1	190 ± 35
ATP 1 mM	7	1584 ± 135	33 0 ± 7.2	510 ± 122	11.1 ± 1.8	162 ± 17	27.0 ± 5.5	130 ± 20|
Pyruvate 10 mM	5	1150 ± 243	7.6 ± 2.7	64 ± 16¶	15.2 ± 4.2	183 ± 38	16.0 ± 1.9	276 ± 75
$PGF_{2\alpha}$ (10^{-6} M) – contracted	6	1002 ± 175	26.0 ± 5.9	262 ± 93	18.3 ± 3.7	167 ± 35	18.3 ± 4.2	203 ± 41
Hemolysate (0.1 g/dl) – contracted	3	1367 ± 302	63.4 ± 15.4§	820 ± 168|	11 3 ± 3.8	120 ± 16	19.5 ± 5.7	219 ± 36

*Contractions relative to those induced by 30 mM K^+; †Decrease in the tension as compared with the level prior to the addition of IAA. N = number of preparations used; ½ D = duration of half the magnitude of contractions; PG = prostaglandin. ‡Significantly different from control, $p < 0.001$; §$p < 0.01$; | $p < 0$ 02; ¶$p < 0$ 05.

n = 3, and 23.0 ± 9.6%, n = 4, respectively) were not significantly different from those seen in control strips (see table 1). Average times to attain the maximum contraction in the IAA-treated preparations were 125 ± 31 and 134 ± 23 min, respectively.

Modification by IAA of the Passive Tension Development

Dog cerebral and mesenteric arterial strips exposed for 60 min to Ca^{++}-free media containing 1 mM EGTA were rapidly stretched by 20 and 40% of the initial length, respectively, and the passive tension development was recorded. Following the stretch, passive tensions rapidly developed, then gradually declined and stabilized at a certain level (fig. 3). Initial and stabilized levels of the developed tension were measured. Cerebral and mesenteric arterial strips exposed for 90 min to 1 mM IAA responded to a rapid stretch with an initial tension development followed by an additional, slowly-developing contraction (fig. 3), which seemed to be the late contraction evoked by the mechanical stretch. Quantitative data are summarized in fig. 4. The initial force responses were not affected but the stabilized responses were significantly increased by treatment with IAA.

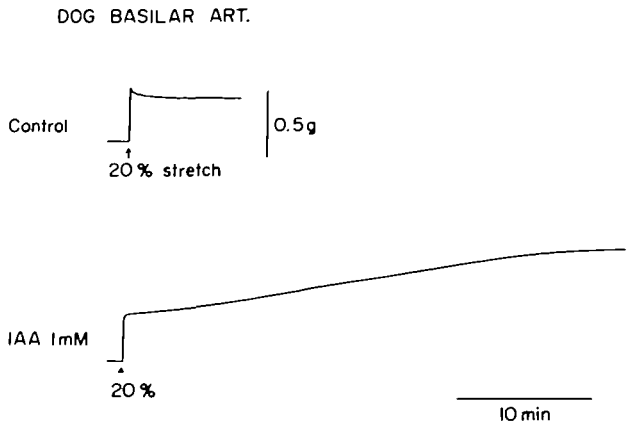

FIGURE 3. *Passive tensions developed by rapid stretch (20% of the initial length) in a dog basilar arterial strip before and 90 min after the addition of 1 mM IAA. The strip had been exposed for 60 min to Ca^{++}-free, EGTA (1 mM)-containing media before the control response was obtained. After the passive tension was stabilized, the stretch was released, then IAA was added.*

Discussion

The addition of IAA caused different patterns of contractions in helically cut strips of a variety of dog arteries; separate contractions of cerebral arteries were induced during an early period and after prolonged exposure, whereas only a late contraction was elicited in mesenteric, coronary, renal and femoral arteries. The magnitude of the early contraction in monkey cerebral arteries was markedly greater than that in dog cerebral arteries. Similar time course of separate contractions has been demonstrated in isolated guinea pig taenia coli.[18] In contrast, isolated bovine mesenteric arteries were reported to be unresponsive to 22 mM IAA.[11]

Only cerebral arteries responded to IAA with an early contraction, which was abolished by exposure to Ca^{++}-free media containing a Ca^{++} chelating agent. Such a treatment also abolishes the contractile response of isolated rabbit aortae to norepinephrine, histamine and angiotensin II.[19] Treatment with verapamil in a concentration (10^{-6} M) sufficient to abolish the contractile response to Ca^{++} of dog cerebral arterial strips exposed to Ca^{++}-free media and depolarized by excess K^+[20] did not significantly reduce the IAA-induced early contraction. On the other hand, dantrolene

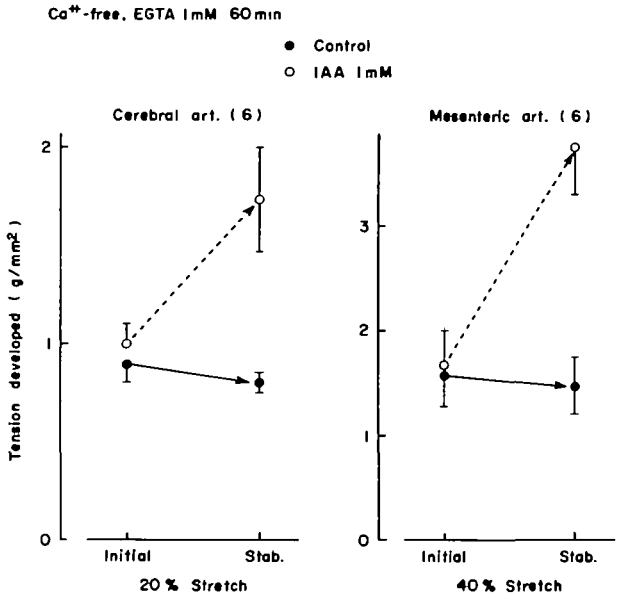

FIGURE 4. *Modification by 1 mM IAA of the passive tension developed by rapid stretch in dog cerebral and mesenteric arterial strips exposed for 60 min to Ca^{++}-free media containing 1 mM EGTA. Initial = passive tension attained immediately after the stretch (20% of the initial length for cerebral artries and 40% of the initial length for mesenteric arteries); Stab. = level of the tension stabilized during an observation period of 10 to 40 min. Vertical bars represent SEM. Numbers in parentheses indicate the number of preparations used.*

(5×10^{-5} M) and procaine (5×10^{-3} M) significantly attenuated the early contraction. These antagonists reportedly interfere with the release of Ca^{++} from intracellularly stored sites.[21-23] In fact, contractions of dog mesenteric arterial strips induced by norepinephrine or prostaglandin $F_{2\alpha}$ are attenuated by the antagonists more preferentially than those induced by excess K^+ (Toda, unpublished data). These results indicate that the mobilization of Ca^{++} from the stored sites is involved in the genesis of IAA-induced cerebroarterial contractions during early periods. Ca^{++} appears to be mobilized more easily in cerebral arterial smooth muscle, particularly of monkeys, than in peripheral arterial muscle.

All the dog and monkey arteries used in the present study contracted following prolonged exposure to IAA. IAA blocks the anaerobic metabolism of glucose by inhibiting the activity of phosphoglyceraldehyde dehydrogenase, thereby decreases the production of pyruvate and lactate, which are utilized in the aerobic tricyclic acid cycle to produce ATP. Treatment with IAA (1 to 5 mM) in association with excess K^+ or fluoroacetate diminishes ATP and creatine phosphate in isolated dog coronary[13] and bovine carotid arteries[24] and rabbit taenia coli.[11] In the IAA (0.5 mM)-treated cat papillary muscle, $^{14}CO_2$ evolution from ^{14}C-glucose is completely inhibited.[25] Arterial contractions induced by prolonged exposure to IAA may thus be considered to result from a depletion of ATP in the vessels. However, the IAA-induced late contraction was not prevented by incubation with ATP or pyruvate nor accelerated under anoxic conditions. Treatment with pyruvate even in a high concentration (50 mM) does not prevent the suppression by IAA (5 mM) of K^+-induced coronary arterial contraction.[13] These findings do not support the idea that a decrease in pyruvate and ATP production is involved in the genesis of IAA-induced late contractions.

Kawai and Brandt[26] have demonstrated that a greater magnitude of contraction develops in skinned crayfish muscle soaked in Ca^{++}-containing solution than in low Ca^{++} medium when ATP is removed. Iodoacetamide (1 mM) or dinitrofluorobenzene (0.38 mM) produces an increased rate of Ca^{++} efflux from isolated frog sartorius muscle which coincides with the development of tension during the early stage of rigor.[15, 27] These phenomena are interpreted to derive from interference with the rebinding of endogenously released Ca^{++} by sarcoplasmic reticulum and, as a result, increase intracellular Ca^{++} levels.[27] This does not seem to occur in dog arterial strips, since the late contraction induced by IAA was not significantly reduced but rather potentiated by removal of external Ca^{++}. Increase in the resting tension of dog tracheal smooth muscle under the rigor state induced by metabolic inhibition is also insensitive to Ca^{++} depletion.[28] Treatment with verapamil, dantrolene, procaine and lidocaine were ineffective in suppressing the IAA-induced late contraction. Further, once the late contraction was established, vasodilator agents did not relax the arteries at all. Thus, involvement of active Ca^{++} in the vicinity of contractile proteins in the induced contraction is if any minimal. Glycerinated skeletal muscle undergoes rigor contraction when deprived of ATP even in the absence of Ca^{++}.[10] In experiments performed in Ca^{++}-free, EGTA-containing solutions, a greater passive tension did not develop under stabilized conditions following a rapid stretch of arterial strips exposed for 90 min to IAA than the tension before the addition of the metabolic inhibitor. Further, the late contraction persisted for only a short period, as compared with rigor contraction seen in skeletal muscle. These findings indicate that arterial smooth muscle does not undergo rigor like that seen in skeletal muscle. Therefore, from the data obtained so far, the mechanism underlying the late contraction induced by IAA cannot be determined.

Delayed cerebral vasospasm in humans is produced with a latency of a few days or longer after subarachnoid hemorrhage.[29] This spasm may therefore be considered to be associated with progressive alterations in the vascular function, possibly due to impaired intravascular circulation, impaired metabolism in vascular smooth muscle, exposure of the smooth muscle to accumulated vasoconstrictor substances, etc. Refractoriness of the spasm to vasodilator therapy let me assume that the mechanism underlying the spasm is not necessarily related to the intracellular accumulation of active Ca^{++}. Such is also postulated here in the genesis of late contractions in the arteries treated with IAA. However, the magnitude and the duration of contractions do not appear to be sufficient to explain the extreme,

persistent narrowing of large vessels clinically seen after subarachnoid hemorrhage. Further, following long exposure to IAA, the reactivity of smooth muscle cells to vasoconstrictor substances was abolished. Reduced reactivity to vasoconstrictor agents has also been shown in middle cerebral arteries isolated from dogs in which experimentally-induced subarachnoid hemorrhage and vasospasm persisted.[30] Therefore, such a muscle contraction induced by active substances cannot be supplemental to the contraction probably induced in cerebral arteries placed under prolonged metabolic inhibition.

Acknowledgements

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