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Fe^{2+}-Induced Lysis and Lipid Peroxidation of Chromaffin Granules

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Abstract: Chromaffin granules, the catecholaminergic storage granules from adrenal chromaffin cells, lysed in 10^{-9}–$10^{-7}M$ Fe^{2+}. Lysis was accompanied by the production of malondialdehyde which results from lipid peroxidation. Both chromaffin granule lysis and malondialdehyde production were inhibited by the free radical trapping agent butylated hydroxytoluene but not by catalase and/or superoxide dismutase. The results suggest that lysis resulted from a direct transfer of electrons from Fe^{2+} to a component of the chromaffin granule membrane without the participation of either superoxide or hydrogen peroxide and may have resulted from lipid peroxidation. In some experiments, ascorbate alone induced chromaffin granule lysis which was inhibited by EDTA, EGTA, or deferoxamine. The lysis was probably caused by trace amounts of reducible polyvalent cation. Lysis sometimes occurred when Ca^{2+} was added with EGTA (10 μM free Ca^{2+} concentration) and was consistently observed together with malondialdehyde production in the presence of Ca^{2+}, EGTA, and 10 μM Fe^{2+} (total concentration). The apparent Ca^{2+} dependency for chromaffin granule lysis and malondialdehyde production was probably caused by a trace reducible polyvalent ion displaced by Ca^{2+} from EGTA and not by a Ca^{2+}-dependent reaction involving the chromaffin granule. **Key Words:** Chromaffin granules—Fe^{2+}—Malondialdehyde—Lipid peroxidation—Ca^{2+}. **Spears R. M. and Holz R. W.** Fe^{2+}-induced lysis and lipid peroxidation of chromaffin granules. *J. Neurochem.* **44**, 1559–1565 (1985).

Secretion of prepackaged hormones and neurotransmitters generally occurs by exocytosis. Studies concerning catecholamine secretion from adrenal medullary chromaffin cells defined the process biochemically and demonstrated that Ca^{2+} influx (Douglas and Rubin, 1961; Viveros et al., 1968; Kilpatrick et al., 1982; Holz et al., 1982) and a rise in cytosolic Ca^{2+} concentration (Knight and Kesteven, 1983) are responsible for initiating exocytosis. In the course of experiments examining the effects of Ca^{2+} on the stability of chromaffin granules, the catecholamine-containing secretory granules from the adrenal medulla, we found that isolated chromaffin granules lysed in the presence of ascorbate and micromolar calcium that was buffered with EGTA. The present work demonstrates that the lysis was caused not by Ca^{2+} but by trace amounts of a reducible polyvalent ion that was displaced by Ca^{2+} from EGTA. The effects could be reproduced in the absence of calcium by nanomolar concentrations of ferrous ion, which probably directly altered chromaffin granule stability by lipid peroxidation. The loss of storage granule stability by low concentrations of Fe^{2+} has both experimental and clinical implications.

MATERIALS AND METHODS

Cells disaggregated from bovine adrenal medulla (Fenwick et al., 1978; Kilpatrick et al., 1980; Holz et al., 1982) were plated at a density of 250,000–500,000 cells/cm^2 in 60-mm diameter uncoated plastic petri dishes (Lux Scientific, Newbury Park, CA, U.S.A.) and in 75-cm^2 and 150-cm^2 plastic culture flasks (Costar, Cambridge, MA, U.S.A.) in minimum essential medium (MEM; Eagle's) (GIBCO, Grand Island, NY, U.S.A.) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 10 μM cytosine arabinoside (to inhibit fibroblast proliferation), 50 μg/ml gentamycin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml Fungizone (Squibb, Princeton, NJ, U.S.A.). There were 1–2 million cells/ml culture medium. By 4 days at 34°C in 5% CO_2-95% air the chromaffin cells had formed monolayers and the in-

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Abbreviations used: MEM, minimum essential medium; PIPES, piperazine-*N*-*N*'-bis(2-ethanesulfonic acid).

cubation medium was replaced with fresh medium. Experiments were performed on days 5–10. There were approximately 40 nmol catecholamine/million cells.

The catecholamine stores of chromaffin cells in 60-mm diameter plastic dishes were labelled with [^{3}H]-norepinephrine by preincubation of the cells in 3 ml of MEM containing [^{3}H]norepinephrine (0.3 μCi/ml, 13 Ci/mmol, Amersham, Arlington Heights, IL, U.S.A.) and 0.1 m*M* ascorbate for 1–2 h (Kilpatrick et al., 1980). The cells were washed twice for 5 min with MEM and then once with isotonic physiological saline solution [145 m*M* NaCl, 5.6 m*M* KCl, 15 m*M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.5 m*M* $MgCl_2$ and 2.2 m*M* $CaCl_2$, 5.6 m*M* glucose, and 0.1 m*M* ascorbate].

Preparation of chromaffin granules

Chromaffin granules were harvested by scraping the cells from the culture dish in 260 m*M* sucrose, 10 m*M* piperazine-*N*-*N'*-bis(2-ethanesulfonic acid) (PIPES; pH 7.0), and 1 m*M* EGTA and homogenizing the suspension in a 7-ml Dounce homogenizer with a tight-fitting pestle with 12 vigorous strokes. The homogenate was centrifuged at 1,000 × *g* for 5 min. The supernatant (S_1) was centrifuged at 40,000 × *g* for 10 min and the pellet (P_2) was resuspended (using a 2-ml Dounce homogenizer) in 260 m*M* sucrose, 10 m*M* PIPES (pH 7.0) buffer to an approximate protein concentration of 0.5 mg/ml.

Highly purified chromaffin granules were prepared by sedimentation of the granules through a sucrose-Ficoll-D_2O solution (Trifaro and Dworkind, 1970). A P_2 fraction was prepared from chromaffin cells which were maintained as a monolayer in a tissue culture flask at a density of approximately 450,000 cells/cm^2 or from fresh adrenal medulla (Holz, 1979). The P_2 pellet was resuspended in 1–2 ml of 260 m*M* sucrose, 10 m*M* PIPES (pH 7.0), layered over 7.5 ml of 19.5% Ficoll, 260 m*M* sucrose, 10 m*M* PIPES (pD 7.0) in D_2O and centrifuged at 100,000 × *g* for 60 min in a Beckman Type 40 rotor. The pellet was rinsed (without resuspension) once with 1 ml of sucrose buffer (325 m*M* sucrose, 10 m*M* PIPES, pH 7.0) and then resuspended in 1 ml of the same buffer using a 2-ml Dounce homogenizer. The concentration of the suspension was adjusted to 0.25–0.5 mg protein/ml before use.

[^{3}H]Norepinephrine release

Chromaffin granule suspensions were aliquoted (10 μl) into 2-ml plastic centrifuge tubes. Incubations were started by addition of 190 μl of solution at 30°C. When malondialdehyde was measured concurrently with catecholamine release, 30 μl of suspension was incubated under the same conditions with 470 μl of solution. Reactions were stopped by addition of EDTA and, occasionally, hydroquinone. The suspensions were centrifuged at 40,000 × *g* for 10 min. Supernatants were transferred to 5-ml plastic scintillation vials containing an equal volume of 2% Triton X-100. Pellets were solubilized with 0.4 ml of 1% Triton X-100 and also transferred to scintillation vials. Scintillation counting cocktail (ACS; Amersham) was added and radioactivity determined. Endogenous catecholamine was measured using a fluorescent assay (Holz et al., 1982).

Measurement of lipid peroxidation

Lipid peroxidation was estimated by measuring the reaction of thiobarbituric acid with malondialdehyde, which is formed from the breakdown of polyunsaturated lipids (Tappel and Zalkin, 1959). Sample (0.45 ml) was mixed with 0.75 ml of 20% acetic acid (pH 3.5), 0.10 ml 8.1% sodium dodecyl sulfate, and 0.75 ml of 0.8% thiobarbituric acid. Tetramethoxypropane dissolved in 325 m*M* sucrose, 10 m*M* PIPES (pH 7.0) was used as a standard. Incubation solutions without tissue were routinely used as blanks. The tubes were capped with marbles and heated in an oven at 85°C for 60 min. The samples were then cooled in a water bath and 0.5 ml of water added to each. The pink thiobarbituric acid-malondialdehyde complex was extracted into 2.5 ml of *n*-butanol-pyridine (15:1) by vigorous vortex-mixing and the phases separated by centrifugation for 5 min at 3,000 r/min in a Sorvall GLC desk-top centrifuge. Absorbance of the organic extracts was measured at 532 nm. Ascorbate (0.1–0.2 m*M*) was added to the acetic acid used in the assay to reduce the background absorbance due to iron.

Other procedures

Ca^{2+} and Fe^{2+} ion concentrations were buffered in the micromolar and submicromolar ranges with 1 m*M* EGTA according to Portzehl et al. (1964). The unbound ion concentrations were calculated using a value for the Ca^{2+}-EGTA dissociation constant of 10^{-11} *M* (Owen, 1976) and a value for the Fe^{2+}-EGTA dissociation constant of $10^{-11.9}$ *M* (Martell and Smith, 1974). Proteins were measured by the method of Bradford (1976).

Chromaffin granule lysis was monitored by measuring decreases in optical density at 430 nm (Johnson and Scarpa, 1976).

Superoxide dismutase activity was measured according to McCord and Fridovich (1969) by the capacity of the enzyme to inhibit the reduction of cytochrome *c* (10 μ*M*) by superoxide which was generated by xanthine oxidase (Sigma, 1.7 mU/ml) and xanthine (50 μ*M*). Catalase activity was measured by the rate of disappearance of H_2O_2 according to Beers and Sizer (1952).

RESULTS

Effects of ascorbate and Fe^{2+} on catecholamine release from chromaffin granules

Ascorbate (10–50 μ*M*) released as much as 90% of the catecholamine from chromaffin granules in a P_2 fraction from primary cultures of adrenal medullary chromaffin cells. The effects of ascorbate were variable and were observed only 60% of the time. The release of catecholamines by ascorbate was blocked by the polyvalent cation chelators EDTA and deferoxamine (Fig. 1), suggesting that catecholamine release was caused by the presence of a contaminating polyvalent cation that was reduced by ascorbate. EGTA (1 m*M*) also inhibited ascorbate-induced lysis. Because ascorbate readily reduces ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) under the conditions used in these experiments, the effect of Fe^{2+} on chromaffin granule stability was investigated. Fe^{2+} could reproducibly mimic the effects of ascorbate when present at very low concentrations (Fig. 2). The species actually responsible for catecholamine release was unbound Fe^{2+} rather than chelated Fe^{2+} since high concentrations of EGTA inhibited the effects of Fe^{2+} (Fig. 2). The

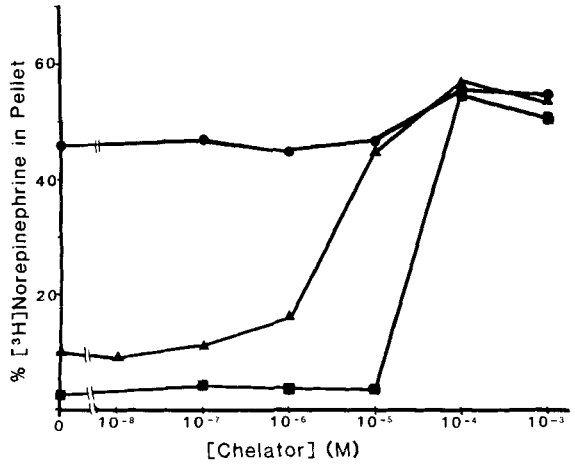

FIG. 1. Effects of EDTA and deferoxamine on ascorbate-induced adrenal chromaffin granule lysis. Granules from a P_2 fraction were incubated at 30°C for 45 min (0.48 mg protein/ml) in 260 m*M* sucrose-10 m*M* PIPES (pH 7.0) with 50 μ*M* ascorbate and various concentrations of EDTA (■) or deferoxamine (▲). Samples were also incubated with various concentrations of EDTA without ascorbate (●). Reactions were terminated by addition of EDTA to 2.5 m*M*. Results are expressed as the percentage of [^{3}H]norepinephrine remaining in the granules as a function of the logarithm of the chelator concentration. There were three samples/group.

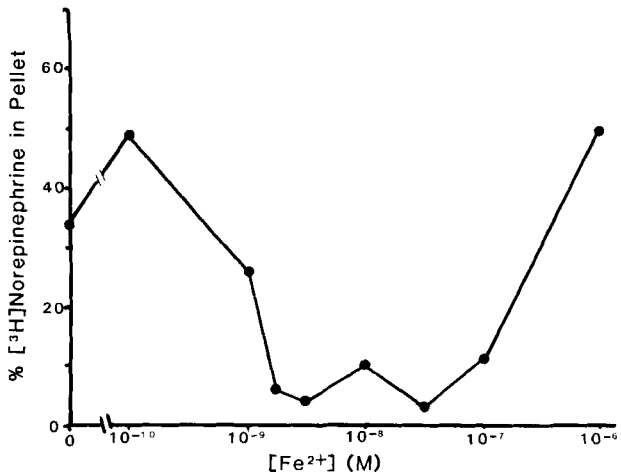

FIG. 2. Effects of various concentrations of unbound Fe^{2+} on chromaffin granule lysis. Purified chromaffin granules (0.52 mg protein/ml) were incubated in 325 m*M* sucrose, 10 m*M* PIPES (pH 7.0) with various concentrations of unbound Fe^{2+} without added ascorbate at 30°C for 60 min. Unbound Fe^{2+} concentrations were maintained using FeEGTA (1 m*M* total EGTA). Reactions were terminated by addition of EDTA and hydroquinone to 4 m*M* and 10 m*M*, respectively. Results are expressed as the percentage of [^{3}H]norepinephrine remaining in the granules as a function of the logarithm of the Fe^{2+} concentration. Results obtained in solutions with EGTA (1 m*M*) without Fe^{2+} and without both EGTA and Fe^{2+} were identical and are represented as 0 Fe^{2+} in the figure. There were three samples/group.

effective Fe^{2+} concentration range was approximately 10^{-9}–10^{-7} *M*. Fe^{2+} concentrations of 10^{-6} *M* or greater did not cause lysis. Most experiments were performed with granules prepared from chromaffin cells maintained as monolayers in culture from 4 to 10 days. Similar results were obtained in one experiment with granules prepared from fresh adrenal medulla.

Granule lysis and lipid peroxidation

Lysis of chromaffin granules can be monitored by measuring the loss of optical density in a suspension of purified granules (Hillarp and Nilson, 1954; Johnson and Scarpa, 1976). Fe^{2+} (21 n*M*) induced a decrease in optical density of a suspension of purified chromaffin granules (Fig. 3). The data indicate that the Fe^{2+}-induced release of catecholamines reflects a time-dependent granule lysis and not a selective permeability increase to catecholamines. Lysis was not observed in the presence of either high concentrations of EDTA (1 m*M*) or Fe^{2+} (10 μ*M* in the absence of added chelator).

Fe^{2+} may destabilize chromaffin granules by

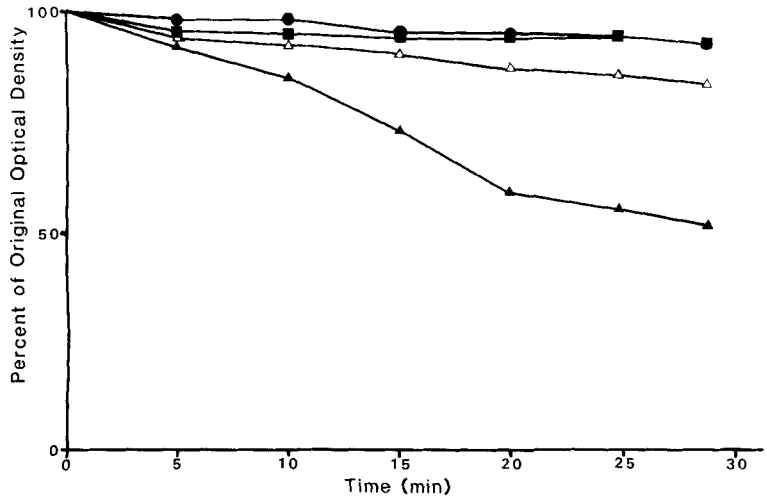

FIG. 3. Time course of Fe^{2+}-induced loss of optical density of a suspension of chromaffin granules. Purified chromaffin granules (0.46 mg protein/ml) were incubated in 325 m*M* sucrose, 10 m*M* PIPES (pH 7.0) without added ascorbate containing either 100 μ*M* Fe^{2+} (●); 100 μ*M* Fe^{2+} + 5 m*M* EGTA (unbound Fe^{2+} concentration 0.65 n*M*) (■); 100 μ*M* Fe^{2+} + 250 μ*M* EGTA (unbound Fe^{2+} concentration 21 n*M*) (▲); or 100 μ*M* Fe^{2+} + 250 μ*M* EGTA (unbound Fe^{2+} concentration 21 n*M*) and 100 μ*M* butylated hydroxytoluene (△). Absorbance at 430 nm (A_{430}) was monitored concurrently for all four suspensions. A_{430} of the suspensions at the beginning of the incubation was 0.22. The optical density of granules lysed in H_2O, to which sucrose buffer was subsequently added, was 0.06 and was subtracted from the total optical densities. Data are expressed relative to A_{430} at 0 time.

transferring electrons to acceptors on the granule membrane (possibly polyunsaturated lipids), thereby generating a free radical that would initiate free radical reactions. Indeed, butylated hydroxytoluene, which is an antioxidant and free radical trapping agent, inhibited the Fe^{2+}-induced granule lysis (Fig. 3 and Table 1).

Lipid peroxidation can be initiated by formation of free radicals in polyunsaturated fatty acid moieties with the subsequent rearrangement of double bonds to form conjugated dimer (Buege and Aust, 1978). Addition of molecular oxygen results in peroxy radicals and subsequent rearrangements to lipid hydroperoxides and lipid endoperoxides. Some of the lipid endoperoxides break down to produce malondialdehyde. Enzymatic and nonenzymatic initiation of the chain reaction is well documented (McKnight et al., 1965; Pederson and Aust, 1975; Buege and Aust, 1978; Sharma, 1979). Fe^{2+} caused the formation of malondialdehyde coincident with granule lysis in a purified granule preparation (Table 1). Superoxide dismutase (83 μg/ml) and/or catalase (66 μg/ml) did not inhibit Fe^{2+}-induced granule lysis or malondialdehyde production (Table 2). Similar results were obtained with a P_2 fraction. The results suggest that neither superoxide nor hydrogen peroxide participated in chromaffin granule lysis induced by Fe^{2+} and are consistent with electrons being transferred directly from Fe^{2+} to the granule membrane to initiate lipid peroxidation and granule lysis. However, it should be noted that although superoxide dismutase activity was unaltered by EGTA ± Fe^{2+} and catalase activity was unaltered by EGTA in the incubations, the effects of EGTA and Fe^{2+} on catalase activity could not be determined (see footnote to Table 2). Thus, it is conceivable that catalase activity was insufficient in the presence of Fe^{2+} to reduce H_2O_2 that may have been generated in the granule experiments.

The data with purified granules suggest that the effects of Fe^{2+} were directly on the granules and not mediated by other cellular components. Because both purified granules and granules in a P_2 fraction behaved similarly and P_2 fractions were more readily prepared, some of the following experiments were performed with a P_2 fraction.

TABLE 1. *Chromaffin granule lysis and malondialdehyde formation*

Treatment	[^{3}H]Norepinephrine in pellet (%)	Malondialdehyde produced (nmol)
0 Fe^{2+} (with 1 m*M* EGTA)	55 ± 1	0
10^{-10} *M* Fe^{2+}	52 ± 2	0
10^{-8} *M* Fe^{2+}	5 ± 2[a]	1.57 ± 0.09[a]
10^{-6} *M* Fe^{2+}	54 ± 1	0.023 ± 0.003[a]
2 × 10^{-8} *M* Fe^{2+} + BHT (200 μ*M*)	53 ± 2	0

Chromaffin granules mg protein/ml) purified from cultured chromaffin cells by sedimentation through Ficoll/D_2O were incubated for 1 h at 30°C in various concentrations of Fe^{2+} in sucrose buffer 325 m*M* sucrose 10 m*M* PIPES, pH 7.0 (without added ascorbate). The free Fe^{2+} was buffered using 1 m*M* EGTA. The reaction was stopped by adding EDTA to 4 m*M*. There were three samples/group.

[a] $p < 0.001$ vs. 0 Fe^{2+} (with 1 m*M* EGTA).

Calcium effects and Fe^{2+}-induced lysis

This entire study began because of the initial observation that chromaffin granules lysed in the presence of micromolar calcium (buffered with 1 m*M* EGTA) (Fig. 4A). Ascorbate (10–600 μ*M*) was also required for the effect. Lysis was variable, however (occurring in 60% of the experiments), which suggested that a reducible contaminant rather than Ca^{2+} was responsible for granule lysis.

We could consistently observe release of catecholamine from chromaffin granules from cultured cells in the presence of 10 μ*M* added Fe^{2+} when Ca^{2+} was buffered in the micromolar range with 1 m*M* EGTA (Fig. 4B). Associated with catecholamine release was a decrease in optical density of chromaffin granule suspensions and malondialdehyde production (data not shown). The apparent Ca^{2+} dependency, however, was probably an artifact caused by the equilibria between EGTA, Ca^{2+}, and Fe^{2+}. In the absence of added Ca^{2+}, EGTA (1 m*M*) reduced the unbound Fe^{2+} concentration to <1 n*M* which was not sufficient to cause granule lysis. The presence of 0.98 m*M* total Ca^{2+} increased the unbound Fe^{2+} concentration to 10–30 n*M* which was sufficient to induce granule lysis. Similar

TABLE 2. *Effects of superoxide dismutase and catalase on Fe^{2+}-induced lysis and malondialdehyde production in a purified chromaffin granule preparation*

Treatment	[^{3}H]Norepinephrine in pellet (%)	Malondialdehyde produced (nmol)
0 Fe^{2+}	46 ± 2	0
10^{-8} *M* Fe^{2+}	6 ± 2[a]	2.0 ± 0.1[a]
10^{-10} *M* Fe^{2+}	45 ± 1	0
0 Fe^{2+} + SOD	47 ± 1	0
+ Cat	45 ± 1	0
+ SOD + Cat	40 ± 1	0
10^{-8} *M* Fe^{2+} + SOD	7 ± 1[a]	2.1 ± 0.1[a]
+ Cat	7 ± 1[a]	2.0 ± 0.2[a]
+ SOD + Cat	5 ± 1[a]	1.9 ± 0.2[a]

A purified chromaffin granule fraction (0.25 mg protein/ml) was diluted 17-fold into 325 m*M* sucrose, 10 m*M* PIPES (pH 7.0) (without added ascorbate) in the presence or absence of Fe^{2+} and the presence or absence of superoxide dismutase (SOD, 83 μg/ml) and/or catalase (Cat, 66 μg/ml). The Fe^{2+} concentration was maintained with a FeEGTA buffer (with 1 m*M* total EGTA). Groups without Fe^{2+} contained 1 m*M* EGTA. Incubations were carried out for 60 min at 30°C and stopped by addition of EDTA to 4 m*M*. There were three samples/group. Superoxide dismutase was not inhibited by EGTA or EGTA + Fe^{2+} in the incubation medium. Catalase also was not inhibited by EGTA in the incubation. Catalase activity in the presence of small amounts of Fe^{2+} could not be assayed because of oxidation of Fe^{2+} by H_2O_2 which was present in the catalase assay.

[a] $p < 0.001$ vs. 0 Fe^{2+}.

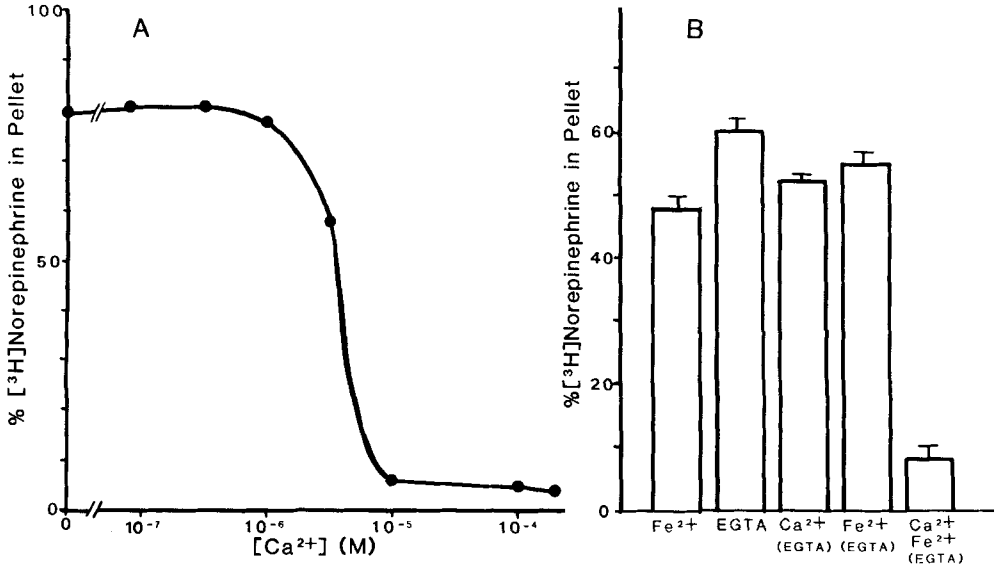

FIG. 4. Apparent Ca^{2+}-dependent release of [^{3}H]norepinephrine from chromaffin granules. **A:** Effects of various Ca^{2+} concentrations on release of catecholamine. A P_2 fraction was diluted 20-fold into 260 m*M* sucrose, 10 m*M* PIPES (pH 7.0), 5 mg/ml bovine serum albumin, 0.6 m*M* ascorbate, 1 m*M* EGTA, and various amounts of Ca^{2+} at 30°C. After 5 min the reaction was stopped by placing the tubes on ice and centrifuging the remaining intact granules at 40,000 × *g* for 10 min. The percent of [^{3}H]norepinephrine in the pellet is plotted as a function of the logarithm of the unbound Ca^{2+} concentration. There were three samples/group. **B:** Requirement of Fe^{2+} for apparent calcium-induced lysis of chromaffin granules. A P_2 fraction was diluted 20-fold into 260 m*M* sucrose, 10 m*M* PIPES (pH 7.0) with various additions as follows: Fe^{2+}, 10 μ*M* Fe^{2+}; EGTA, 1 m*M* EGTA; Ca^{2+}, 10 μ*M* unbound Ca^{2+} buffered with 1 m*M* EGTA; Fe^{2+}, 10 μ*M* added Fe^{2+} + 1 m*M* EGTA, unbound [Fe^{2+}] = 0.3 n*M*; Ca^{2+}, Fe^{2+}, 10 μ*M* unbound Ca^{2-} buffered with 1 m*M* EGTA + 10 μ*M* added Fe^{2+}, unbound [Fe^{2+}] = 10–30 n*M*. Incubations were carried out for 60 min at 30°C in the absence of ascorbate. Reactions were stopped by addition of EDTA to 2.5 m*M* and centrifugation of the remaining intact granules at 40,000 × *g* for 10 min. Data are expressed as percent [^{3}H]norepinephrine remaining in the pellet. There were three samples/group. In this preparation 50 μ*M* ascorbate caused release of approximately half of the granular catecholamine within 60 min (data not shown). The ascorbate-induced release was totally inhibited by 1 m*M* EGTA.

but variable results were obtained using chromaffin granules prepared from fresh adrenal medulla.[1]

Various factors altered granule lysis induced either with ascorbate alone, or with ascorbate and 10^{-5} *M* calcium (buffered with 1 m*M* EGTA). High medium concentrations of ascorbate (e.g., 5 m*M*) or epinephrine (>100 μ*M*), both of which are present in chromaffin granules, prevented granule lysis. Bovine serum albumin also occasionally inhibited ascorbate-induced granule lysis.

DISCUSSION

We have demonstrated that Fe^{2+} (10^{-9}–10^{-7} *M*) in vitro caused lysis of chromaffin granules, the secretory granules from the adrenal medulla. Micromolar (or greater) concentrations of Fe^{2+} did not cause lysis. Lysis was associated with the production of malondialdehyde which is a product of lipid peroxidation. Both granule lysis and malondialdehyde production were inhibited by the free radical trapping agent butylated hydroxytoluene but were not altered by superoxide dismutase and/or catalase. It is possible that lysis resulted from a direct transfer of electrons from Fe^{2+} to a component in the chromaffin granule membrane without the participation of either superoxide or hydrogen peroxide. However, we cannot rule out the possibility that membranes or substances contaminating the purified granule preparations were also involved. The evidence is consistent with lysis being caused by lipid peroxidation. The inability of micromolar Fe^{2+} to cause granule lysis or malondialdehyde production may have resulted from free radical quenching effects of high concentrations of Fe^{2+} (Gould, 1959).

Recently it was demonstrated that liver lysosomes are also susceptible to Fe^{2+}-induced lipid peroxidation and lysis. The effects of Fe^{2+} did not involve reduced oxygen intermediates (Mak et al., 1983) and the mechanism may be similar to that which caused chromaffin granule lysis in the present experiments.

[1] In two of three preparations of chromaffin granules purified from fresh adrenal medulla, there was a Ca^{2+}-dependent (0.98 m*M* total Ca^{2+}, approximately 10 μ*M* free Ca^{2+} concentration) catecholamine release or a decrease in optical density in the presence of Fe^{2+} (10 μ*M* total), EGTA (1 m*M* total), and ascorbate (50 μ*M*). The lack of effect in one experiment may have been caused by high concentrations of inhibitory substances such as ascorbate, which is found in higher concentrations in fresh adrenal medulla than in cultured chromaffin cells (Diliberto et al., 1983).

An apparent Ca^{2+} dependence for granule lysis was sometimes observed in the presence of EGTA and ascorbate and was consistently observed when Fe^{2+} (10–600 μM total concentration) was present. Lysis in the presence of Ca^{2+} probably resulted from displacement of contaminating reduced polyvalent ion from EGTA to yield a concentration of reduced ion in the concentration range adequate for granule lysis and lipid peroxidation. We have not identified the ion. There are numerous reports describing a similar calcium-induced lysis of secretory vesicles (Pinchasi et al., 1979; Kuo et al., 1979; Konings and DePotter, 1982). In these studies, vesicle lysis was observed in the presence of 10^{-5} M free calcium (buffered with EGTA). Low levels of ascorbate (1–10 μM) were also sometimes present. Our results suggest that calcium-induced storage vesicle lysis in solutions in which Ca^{2+} is buffered by polyvalent ion chelators may be caused by contaminating Fe^{2+} or other polyvalent ions that can initiate free radical reactions.

We have no evidence that the Fe^{2+}-induced lysis of chromaffin granules is related to exocytosis. No detectable malondialdehyde is released upon catecholamine secretion from chromaffin cells and the free radical scavenger butylated hydroxytoluene does not alter secretion (R. M. Spears and R. W. Holz, unpublished observations). However, chromaffin granules contain large amounts of reducing equivalents in the form of ascorbate (10 mM), and the granule membrane contains cytochrome b_{561} which can conduct electrons across the membrane (Njus et al., 1983). It is possible that the effects of Fe^{2+} on chromaffin granule stability are related to the redox system of chromaffin granules.

Lipid peroxidation is one of the primary causes of tissue damage in ischemia. In the heart, ischemia and lipid peroxidation are associated with the release of norepinephrine from sympathetic nerves (Rao and Mueller, 1983). The present study raises the possibility that the release of norepinephrine results, in part, from a lipid peroxidation-induced loss of storage vesicle stability. Fe^{2+} also induces lipid peroxidation in vivo in the CNS (Triggs and Willmore, 1984). Thus, it is possible that CNS hemorrhage could release neurotransmitters from storage granules within CNS neurons because of the release of Fe^{2+} from extravasated red blood cells and subsequent lipid peroxidation.

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