Inhibition of Mn^{++}-Catalyzed Autoxidation of Adrenaline by Ascorbic Acid

In various tissues a Ca^{++} inward current is believed to occur during the action potential[1-4], and manganese (Mn^{++}) has been shown to reduce the permeability of the cell membrane to Ca^{++}[1,5,6]. On the other hand, the inward current of Ca^{++} is increased in the presence of adrenaline[2,7]. Recently, the effect of Mn^{++} on the action of adrenaline has been tested in cardiac and smooth muscle preparations[7-10]. It was found that Mn^{++} inhibited the action of adrenaline and this inhibition was explained by an antagonism between Mn^{++} and adrenaline on Ca^{++} permeability. However, it has to be considered that Mn^{++} like other heavy metals[11] might have accelerated the autoxidation of adrenaline and therefore adrenaline became less effective. In the present study we have investigated quantitatively the Mn^{++}-catalyzed autoxidation of adrenaline and its inhibition by ascorbic acid.

Mn^{++} was used as $MnCl_2$. Adrenaline was determined fluorimetrically after its oxidation to adrenochrome with potassium ferricyanide and subsequent rearrangement and stabilization with 5 *N* sodium hydroxide plus ascorbic acid to give adrenolutine[12]. The recovery of adrenaline from a sample was calculated by means of an 'internal standard', a 'reversed blank' and a 'reagent blank'. To the aliquot of the sample taken for the 'reversed blank' the potassium ferricyanide was added 2 min after 5 *N* NaOH plus ascorbic acid. The 'reagent blank' was prepared using all the reagents for the fluorescence assay in the right order but omitting the sample. Under our experimental conditions the fluorescence intensity of the two different blanks should be identical as long as the adrenaline contained in the sample used for the 'reversed blank' was not transformed to a fluorescent product before the addition of 5 *N* NaOH.

Table I shows the effect of 0.1 m*M* Mn^{++} on the recovery of 50 ng adrenaline added to 1 ml of Tyrode solution which was vigorously bubbled with a mixture of 95% O_2 and 5% CO_2, or with N_2, for 0–20 min. When in the absence of Mn^{++} Tyrode solution containing adrenaline was gassed with O_2/CO_2 for 20 min, a progressive decrease of the recoveries of adrenaline from 84.4 to 64% was noted. This decrease was much more pronounced in the presence of Mn^{++}: the recovery was 19.4% after 10 min and 15.4% after 20 min of gassing. With N_2, the recoveries of adrenaline in the presence of Mn^{++} did not differ greatly from those obtained in the absence of Mn^{++}. These results indicate that Mn^{++} catalyzes the autoxidation of adrenaline. Furthermore, it was found that in the above-mentioned cases of decreased recoveries the instrumental values of the 'reversed blanks' were increased. Based on the assumption that adrenochrome is the fluorescent product detected in the 'reversed blanks', the amount of adrenochrome in the sample was calculated by using the difference between the readings for the 'reversed' and the 'reagent blank', respectively. The amount of adrenochrome was expressed as percent of the adrenaline added to the Tyrode solution ('internal standard'). It was found that the percentage of adrenaline recovered (Table I, A) and the amount of adrenochrome (Table I, B) formed in the incubation medium were inversely correlated; the sums of both figures were close to 100% (Table I, C).

In a second series of experiments (Table II) the inhibition of Mn^{++}-catalyzed autoxidation of adrenaline by ascorbic acid was demonstrated. In order to avoid the interference of ascorbic acid, added to Tyrode solution in some experiments, with potassium ferricyanide during the oxidation procedure, adrenaline was separated from the other constituents of the Tyrode solution by adsorption on, and elution from, alumina before the fluorimetric estimation[13]. Tyrode solution (40 ml) containing adrenaline (10^{-7} g/ml) was vigorously bubbled with 95% O_2 plus 5% CO_2 for 0–20 min. Immediately before and after 1, 10, or 20 min of gassing the content of adrenaline was estimated. In the absence of Mn^{++} and in the presence of ascorbic acid (10^{-5} and 5×10^{-5} g/ml), the recoveries of adrenaline ranged from 80.5 to 94.2%. In the absence of ascorbic acid the recovery of adrenaline was only 57.5% after 20 min of gassing. Similar data have been obtained during the first series of experiments (Table I) although the experimental conditions differed in some points (concentration of adrenaline, adsorption procedure). In the absence of ascorbic acid, Mn^{++} caused a complete degradation of adrenaline (Table II). It was also found (not listed in Table II) that the readings of the 'reversed blanks' were low and indistinguishable from those obtained in the absence of Mn^{++}. This indicates a breakdown of adrenaline beyond adrenochrome or adrenolutine to non-fluorescent compounds. This strong degradation, which was not observed in the first series of experiments,

Table I. Effect of Mn^{++} on the autoxidation of adrenaline

		No Mn^{++}			Mn^{++} (0.1 m*M*)		
Time of gassing (min)		0	10	20	0	10	20
O_2/CO_2	A	84.4	77.0	64.0	70.6	19.4	15.4
	B	7.8	20.2	36.8	27.4	88.8	93.0
	C	92.2	97.2	100.8	98.0	108.2	108.4
N_2	A	–	68.2	64.6	–	63.8	64.2
	B	–	27.4	23.0	–	39.8	39.6
	C	–	95.6	87.6	–	103.6	103.8

Data are expressed as % of adrenaline (50 ng) added to 1 ml of Tyrode solution for 20 min. During this 20-min period the solutions were either not bubbled (0 min) or bubbled with a mixture of 95% O_2 and 5% CO_2, or N_2, for 10 or 20 min. A, recovery of unchanged adrenaline. B, amount of adrenaline oxidized to an adrenochrome-like substance. C, sum of A and B. Given are results of single experiments. For details see text.

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Table II. Effect of ascorbic acid in protecting adrenaline from autoxidation in the presence, or absence, of Mn^{++}

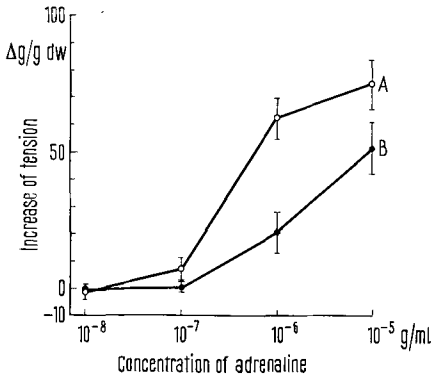
	No Mn^{++}		Mn^{++} (0.1 mM)			
Time of gassing (min)	0	20	0	1	10	20
No ascorbic acid	76.0	57.5	1.4	0.0	2.0	0.0
Ascorbic acid (10^{-5} g/ml)	85.0	81.0	67.5	35.8	13.7	7.3
Ascorbic acid (5×10^{-5} g/ml)	94.2	80.5	97.0	87.0	99.2	90.5

Data are recoveries (%) of adrenaline (4 µg/40 ml Tyrode solution). For details see legend of Table I and text.

Table III. Increase of isometric tension development of electrically driven left atria of guinea-pigs by adrenaline

	A	B
Increase of isometric tension	378.5 ± 76.4 (n = 10)	188.4 ± 47.7 (n = 10)

The Tyrode solutions containing adrenaline (10^{-6} g/ml) were gassed with a mixture of 95% O_2 and 5% CO_2 for 20 min before their application to the atria. Tension development was measured 5 min after application of the test solutions. A, adrenaline in the presence of ascorbic acid (5×10^{-5} g/ml). B, adrenaline in the presence of Mn^{++} (0.1 mM) and in the absence of ascorbic acid. Data are expressed as percent increase above control (mean ± S.E. of mean); n = number of preparations.



Effect of adrenaline on isometric tension development of electrically driven left atria of guinea-pigs. Ordinate, increase of tension in g/g dry weight (dw) above control (control = tension before addition of adrenaline: 22.8 ± 4.95 g/g dw (A) and 7.1 ± 1.41 g/g dw (B)). Abscissa, concentration of adrenaline (g/ml). Tension development was measured 5 min after addition of adrenaline. Stimulation frequency: 180/min. A, adrenaline in the presence of ascorbic acid (5×10^{-5} g/ml). B, adrenaline in the presence of Mn^{++} (0.1 mM) and in the absence of ascorbic acid. Cumulative application of adrenaline. The figures (n = 12) are expressed as mean ± S.E. of mean. In these experiments the Tyrode solutions containing adrenaline were not gassed before their application to the atria.

is perhaps caused by the experimental conditions prevailing during the adsorption procedure (pH 8.8; heavy stirring for 5 min) which may facilitate the Mn^{++}-catalyzed oxidation process. Addition of ascorbic acid (10^{-5} g/ml) to the Tyrode solution before gassing with O_2/CO_2 partially abolished the rapid breakdown of adrenaline in the presence of Mn^{++}. During 20 min of gassing the recoveries declined gradually from 67.5 to 7.3%. However, 5×10^{-5} g/ml ascorbic acid protected adrenaline completely from its Mn^{++}-catalyzed autoxidation.

In a third series of experiments the positive inotropic effect of adrenaline plus ascorbic acid and of adrenaline plus Mn^{++} without ascorbic acid has been studied in isolated left guinea-pig atria (Figure). In the presence of Mn^{++} the positive inotropic action of adrenaline was only partially inhibited. As shown in Table III, adrenaline, too, had lost only about 50% of its positive inotropic activity when the adrenaline containing Tyrode solution before application was gassed for 20 min with O_2/CO_2 in the presence of 0.1 mM Mn^{++}. In this solution no adrenaline could be detected fluorimetrically, however, 93% of adrenaline had been converted to an adrenochrome-like compound (calculated from the 'reversed blank'). Under our conditions, the lower limit of the fluorimetric estimation of adrenaline was 5×10^{-8} g/ml. Since this concentration had only a slight positive inotropic effect, if any (Figure), one can assume that this oxidation product itself has some positive inotropic activity. In conclusion, the present results demonstrate that the Mn^{++}-facilitated autoxidation of adrenaline has to be considered, when actions of adrenaline are investigated in the presence of Mn^{++}. However, degradation of adrenaline catalyzed by Mn^{++} (0.1 mM) can be abolished by ascorbic acid (5×10^{-5} g/ml) which itself does not influence the contractility of isolated guinea-pig atria[8].

Zusammenfassung. Die Autoxydation von Adrenalin in Anwesenheit von Mn^{++} wurde quantitativ untersucht. Es ergab sich, dass die durch 0,1 mM Mn^{++} bewirkte vollständige Oxidation von Adrenalin durch 5×10^{-5} g/ml Ascorbinsäure verhindert werden konnte.

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Morphological Changes in the Pineal Gland of the Albino Rat by Hypophysectomy and Ovariectomy

Little information is available on the relationship of the ovary or the pituitary to the pineal, although attention has been paid to the function of the pineal gland by demonstration of higher concentration of characteristic biogenic amines in this organ[1,2]. This paper describes morphological changes in the pineal gland of hypophysectomized or ovariectomized rats.

Material and methods. 15 female rats of the Wistar strain aged 9–12 weeks were divided into 3 groups and kept for 14 h in light and 10 h in darkness at 24 h periods throughout the experiments. One week later, 8 rats were hypophysectomized[3] and 3 rats were ovariectomized. The pineals were removed under ether anesthesia 3–6 weeks after the operation and were fixed in gultaraldehyde solu-