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THE PHARMACOLOGY OF KCN AS A PROPHYLACTIC AGAINST RADIATION*

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Abstract—A dose of 100 μg of KCN protects about 80 per cent of mice against a lethal dose of total body irradiation; on lowering the dose the protection decreases rapidly. A protective dose of KCN markedly reduces the oxygen tension in the spleen and bone marrow of these mice. The same dose reduces the total oxygen consumption of the animal to about 25 per cent. The respiration is slowed considerably but the respiratory volume shows little change. The oxygen saturation of the arterial blood is unaltered, the saturation of the venous blood is increased. After an initial fall with about 50 mm Hg the blood pressure rises again but remains about 20 mm below normal.

Concurrently with the hypoxia in the spleen and bone marrow the oxygen tension in the brain is augmented considerably. It is concluded that KCN protects against radiation by causing hypoxia in the blood-forming organs. This hypoxia is probably caused by a severe local vasoconstriction due to intense vasomotor stimulation.

INTRODUCTION

IN A previous paper[1] it was shown that a number of biologically active amines which protected mice against a dose of X-rays which otherwise would kill the animals by inactivation of the blood-forming organs, decreased the oxygen tension in the spleen of these mice at protective doses. As a relation was found between the percentage survival of the mice and the percentage reduction of the oxygen tension in the spleen it was concluded that the protection afforded by these compounds was caused by their ability to lower the oxygen tension in the spleen, and possibly also in other blood-forming organs, thereby rendering these organs less sensitive to radiation. This does not imply that all protective compounds act by this same mechanism. In order to explain the prophylactic effect of cyanide a number of hypotheses were put forward.

Bacq *et al.*[2] who discovered the prophylactic effect of cyanide in mice, at first assumed that it acted either by decreasing the metabolic activity or by inhibition of peroxydase. Later Alexander *et al.*[3] suggested that the effect of cyanide might be explained by inactivation of HO_2 radicals.

Cohen *et al.*[4] postulated that the inhibition of cytochrome oxidase by cyanide might be responsible for its protective activity. They assumed that due to this inhibition some link in the metabolic chain, representing the radiosensitive site, was kept in the reduced form and was therefore less sensitive to radiation.

Recently van den Brenk and Moore[5] suggested that cyanide might protect by respiratory inhibition followed by anoxic anoxaemia.

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If inhibition of cytochrome oxidase in the tissues were the important feature one might expect that cyanide can protect mice while the oxygen tension in the blood-forming organs is normal or even increased. This would have interesting theoretical implications since, because of the highly specific action of cyanide, the radiosensitive site would then be open to direct biochemical investigation.

However, our experiments showed that KCN injected into mice at protective doses lowered the oxygen tension in the spleen to a sufficient extent to explain its action by a mechanism identical with that of the biologically active amines. Further experiments to be described in this paper were directed towards the elucidation of the mechanisms by which this reduction in oxygen tension was caused in our mice at doses that protected these animals against irradiation.

METHODS

Experimental animals

All animals used were females of our highly inbred CBA/Rij strain weighing 18–20 g.

Irradiation and estimation of protective activity*

This was performed in exactly the same way as described in a previous paper.[1] All animals received 675 r, a dose which causes over 98 per cent mortality in the control animals. Protective activity was expressed as the percentage 30 days' survival.

Estimation of the oxygen tension in the spleen, brain and bone marrow

Oxygen tension in the spleen was estimated as described in a previous paper.[1] In order to estimate the oxygen tension in the brain a 100-μ platinum wire was pushed through an opening in the skull into the right hemisphere over a distance of 1–2 mm. The localization of the electrode was not standardized. The electrode was connected to the surface of the skull by dentist's cement (Justi Resin Cement) and exteriorized on the back of the neck. The oxygen tension in the bone marrow was estimated by pushing a 50μ platinum electrode into the right femur after a small hole was drilled in the bone between the upper and middle third of the shaft. The electrode was pushed into the marrow cavity over a distance of 2 mm. The electrode was fastened by a thread around the femur just above the hole. It was exteriorized at the back of the mouse.

Determination of the oxygen consumption of the intact animal

The oxygen consumption was determined in a closed system. The changes in volume were measured every minute at atmospheric pressure using a 50-ml burette filled with water. A special device was necessary to inject the animal with KCN while in the closed system. This apparatus will be described in detail elsewhere. In principle it consists of part of a plastic syringe connected by means of Sterivac no. 1 polythene tubing to the peritoneal cavity. The plunger of this syringe is pulled in by two rubber bands. The rubber bands are stretched by means of a small piece of a match placed between a knob on the plunger and a Perlon thread between two small pillars on the

* We are grateful to Dr. D. W. van Bekkum for performing the irradiation experiments. Recent measurements by Dr. Joh Blok have shown that the radiation doses presented in this and previous papers must be multiplied by 1·14 in order to obtain the midline dose in roentgens for mice. This corrected dose is considered to differ less than 4 per cent from the tissue dose. We prefer to maintain our original dose value in this paper for the convenience of comparing our present results with those presented in previous papers.

end of the syringe. Around one of these pillars a 100-μ platinum wire is wound which is connected to a copper antenna. When a high-frequency signal is applied from the outside the platinum wire is heated, the Perlon thread melts and the plunger is released. This device is fastened to the back of the animal and the canula brought into the peritoneum under light ether anaesthesia at least 1 hr before the experiment. Apparently the animal is not in the least disturbed by the presence of this apparatus on its back as it does not try to remove it and behaves quite normally.

Determination of the respiratory volume

Under urethane anaesthesia (0·5 g/kg intraperitoneally, 0·5 g/kg subcutaneously) a canula consisting of a piece of nylon tube N_1 (Talas, Zwolle, Holland) was put into the trachea. This canula was connected to a glass vessel containing 290 ml of air. The pressure variations in this container were recorded with a pressure transducer (capacitive displacement meter, Van Reysen, Delft, Holland) and an Ediswan pen writer. The air in the container was changed every 5 min. The curves were magnified photograpically about five times and the surface under the curve measured with a planimeter over a distance corresponding to 4 sec. As only changes in respiratory volume were of interest the respiratory volume is expressed as cm^2/min. The respiration frequency could be read directly from the curve.

Determination of the oxygen saturation of the blood

Either the left carotic artery or the right jugular vein were canulated in animals under urethane anaesthesia (0·5 g/kg intraperitoneally, 0·5 g/kg subcutaneously). The arterial canula consisted of a piece of Sterivac no. 1 drawn out at the tip over a small piece of no. 20 hypodermic needle. The venous canula was just a piece of Sterivac no. 1.

Before the experiment 0·1 ml of heparin Vitrum (diluted to 67 i.u./ml) was injected through the canula. Heparin Vitrum (5000 i.u./ml) was diluted twenty times with a saponin solution (Merck) containing 0·2 g/ml in distilled water. This solution was brought to the 0·05-ml mark into a 1-ml tuberculin syringe containing two small glass spheres. After a few drops of blood were drawn from the canula the syringe was filled with blood until the solution reached the 0·2-ml mark. The contents were mixed well by means of the glass spheres. Then 0·1 ml was removed and the remainder poured into a Perspex cuvette with a light path of about 0·2 mm. The density at 560 mμ and at the isobestic point 505 mμ was read in a Beckman model B spectrophotometer and the ratio D560/D505 calculated. As the results from the experiments were perfectly clear in themselves no determinations of fully oxygenated and fully reduced blood were made which would enable the exact calculation of the percentage oxygen saturation corresponding with each ratio of D560/D505. For comparison it may be mentioned that for rat blood a difference of 0·1 in this ratio corresponds to a difference of about 10 per cent in the oxygen saturation.

Determination of the blood pressure

For the determination of the blood pressure in the unanaesthetized mouse the same method was used as described previously.[1] An "average blood pressure" was recorded by using a slow recording galvanometer.

KCN

Fresh solutions of KCN in distilled water were made up immediately before each experiment. These solutions were not neutralized as even careful neutralization with HCl caused considerable variation in the cyanide content.

RESULTS

The protection of KCN *against radiation*

Three doses of KCN were injected intraperitoneally into groups of mice just before irradiation with a lethal dose of X-rays (675 r). The effect of this treatment on the 30-days' survival is presented in Table 1.

TABLE 1. THE EFFECT OF KCN INJECTED INTRAPERITONEALLY JUST PRIOR TO IRRADIATION OF FEMALE CBA/RIJ MICE OF ABOUT 20 G WITH A DOSE OF 675 R OF X-RAYS

Dose (μg)	Number of mice	30-days' survival	Percentage protected
100	70	57/70	81
75	40	12/40	30
50	40	3/40	8
—	40	0/40	0

Table 1 shows that 100 μg of KCN, which is very close to a lethal dose, gives good protection. At lower doses the protection decreases rapidly.

The effect of KCN *on the oxygen tension in the spleen and bone marrow*

The same doses of KCN were used to study the effect of KCN on the oxygen tension in the spleen of unanaesthetized mice. The current flowing at 0·6 V between a 100 μ platinum electrode in the spleen (negative side) and a silver–silver chloride electrode in the rectum was measured before and after the administration of KCN. Percentage reduction of oxygen tension was calculated as

$$100 - \frac{\text{current after KCN}}{\text{current before KCN}} \times 100$$

The time between the intraperitoneal injection and the moment at which the current had returned to its preinjection value was also noted. The results are shown in Table 2.

TABLE 2. THE EFFECT OF KCN ON THE OXYGEN TENSION IN THE SPLEEN OF UNANAESTHETIZED FEMALE CBA/RIJ MICE OF ABOUT 20 G

Dose (μg)	Number of mice	Average maximal reduction of oxygen tension (% $\pm$ s.d.)	Duration of the effect (min $\pm$ s.d.)
100	17	82 $\pm$ 3·3	30 $\pm$ 2·9
100*	9	83 $\pm$ 4·7	24 $\pm$ 2·6
75	11	84 $\pm$ 4·3	21 $\pm$ 2·0
50	13	72 $\pm$ 5·0	15 $\pm$ 1·9

* KCN injected 20 min after 1 g/kg urethane (0·5 g intraperiteoneally, 0·5 g subcutaneously).

Doses of 100 and 75 μg per animal reduce the oxygen tension by about 80 per cent, and the effect of 50 μg is possibly somewhat smaller. Although the magnitude of the effect is not much different for the three doses used, the duration of the effect decreases considerably with the dose. As the time interval between the injection and the end of the irradiation lies between 17 and 20 min in our irradiation experiments, the duration of the effect may become a significant feature determining the amount of protection. The group receiving 100 μg of KCN under urethane anaesthesia was added because a number of experiments to be described below could not be performed on unanaesthetized animals and were made under urethane anaesthesia. In eight unanaesthetized mice receiving 100 μg of KCN the oxygen tension in the bone marrow was estimated. The average maximal reduction of the oxygen tension was 78 $\pm$ 4·4 per cent and the average duration of the effect 24 $\pm$ 1·9 min, in good correspondence with the effect in the spleen.

Although we do not claim that our measurements are quantitative our impression is that the oxygen tension in the bone marrow is not significantly lower than in the spleen. The relatively low values found by Cater and Silver[6] in normal human bone marrow may be explained by the difficulties of the technique, differences in the site of placements of the electrode or differences in the animal species investigated.

The effect of KCN *on the oxygen consumption of the intact animal*

The three doses of KCN were used for studying their effect on the oxygen consumption of the intact animal. The oxygen uptake was measured every minute. The average uptake in ml per min at 0 °C and 760 mm Hg over periods of 10 min was calculated for each animal. Means and standard deviations of the 10-min averages for the animals in each dose group are presented in Table 3.

Table 3 shows that a marked reduction of the oxygen consumption down to about 25 per cent of the control value is caused by 100 μg of KCN. Even 80 min after the injection the oxygen consumption is still below normal. For the lower doses of KCN the inhibition of the oxygen consumption is somewhat smaller but especially the duration of the effect is much shorter.

The effect of KCN *on respiratory volume and frequency of respiration*

In order to perform our pharmacological experiments under conditions as closely similar as possible to those under which the animals were irradiated it was attempted to derive the respiratory volume from measurements of intrapleural pressure in the unanaesthetized mouse. This method does not offer too many technical difficulties, but a comparison of the changes in intrapleural pressure with pressure changes in a closed system connected to the trachea under urethane anaesthesia, showed that the relation between the values obtained with both methods which was constant in normal animals, differed considerably after the injection of KCN. Therefore our experiments had to be performed on animals in urethane anaesthesia in which the trachea was canulated. As it was shown in Table 2 that urethane anaesthesia has little effect on the changes in oxygen tension in the spleen the results of experiments under urethane anaesthesia appear to give adequate information. The effect of 100 μg of KCN on respiratory volume and respiration frequency are presented in Tables 4 and 5. These tables show a very marked decrease in respiration frequency to less than a third of the original value accompanied by somewhat erratic changes in respiratory volume. Only one of the four experiments shows a considerable reduction of the respiratory volume.

TABLE 3. THE AVERAGE OXYGEN CONSUMPTION (ML/MIN) OF FEMALE CBA/RIJ MICE OF ABOUT 20 G BEFORE AND AFTER INTRAPERITONEAL INJECTION OF KCN

(Averages of 10-min periods.)

KCN (μg)	Number of mice	Average oxygen consumption over 10-min periods ± s.d.									
		Before KCN		After KCN							
		20–10	10–0	0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80
100	5	1·72 ± 0·04	1·72 ± 0·07	0·41 ± 0·04	0·38 ± 0·03	0·45 ± 0·06	0·60 ± 0·11	0·71 ± 0·19	0·77 ± 0·20	0·92 ± 0·18	1·11 ± 0·07
75	4	1·56 ± 0·03	1·50 ± 0·05	0·51 ± 0·01	0·64 ± 0·04	0·85 ± 0·11	1·22 ± 0·17	1·39 ± 0·05	1·39 ± 0·02	—	—
50	6	1·52 ± 0·09	1·47 ± 0·10	0·58 ± 0·03	1·02 ± 0·11	1·29 ± 0·17	1·39 ± 0·11	1·35 ± 0·12	1·43 ± 0·10	—	—

It should be noted that the variation in the values for the normal respiratory volumes is caused by differences in the setting of the recording apparatus.

The effect of KCN *on the oxygen saturation of arterial and venous blood*

The oxygen saturation was determined as the ratio of the optical density at 560 mμ over the density at 505 mμ (D560/D505) in haemolysed blood. Only two

TABLE 4. INSPIRATORY VOLUME (IN CM^2/MIN, SEE METHODS) BEFORE AND AFTER INTRAPERITONEAL INJECTION OF 100 μG KCN

Female CBA/RIJ mice about 20 g. urethane 1 g/kg (0·5 g intraperitoneally, 0·5 g subcutaneously)

Mouse no.	Respiratory volume (cm^2/min)			
	Before	2 min after	5 min after	10 min after
1	150	225	128	—
2	405	210	195	225
3	360	360	375	630
4	285	375	210	525

TABLE 5. RESPIRATION FREQUENCY OF THE MICE OF TABLE 4

Mouse no.	Respirations per min			
	Before	2 min after	5 min after	10 min after
1	180	75	45	—
2	210	105	75	150
3	285	60	60	75
4	210	180	60	150

determinations were made on each animal either in arterial or in venous blood. One determination was made before KCN, the other at different intervals after intraperitoneal injection of 100 μg of KCN as shown in Table 6.

It is apparent from Table 6 that the saturation of the arterial blood is practically unaltered whereas the saturation of the venous blood is increased as shown by a decreased ratio D560/D505. Therefore there is no reason to assume that the decrease in oxygen tension in the spleen is caused by insufficient oxygenation of the blood.

The effect of KCN *on the blood pressure*

The blood pressure was recorded in unanaesthetized mice. Four doses of KCN were used. The blood pressure after KCN was compared with the value before KCN and the difference in mm Hg averaged over the group of mice. The results are presented in Table 7.

A dose of 25 μg has very little effect except for a small rise in blood pressure. The higher doses produce an initial fall of about 50 mm Hg after which the pressure very soon either returns to normal (50 μg) or remains for some time about 20 mm below the pre-injection level. During these experiments the heart rate was also recorded. However, the results are so erratic that it is impossible to draw any conclusion from them.

It is felt that the relatively small fall in blood pressure apparent from the experiments of Table 7 is not sufficient in itself to cause the considerable reduction of oxygen tension noted in the spleen. Presumably this can only be caused by vasoconstriction either as a compensation for the fall in blood pressure or as a result of stimulation of the vasomotor centre by KCN itself.

TABLE 6. THE EFFECT OF INTRAPERITONEAL INJECTION OF 100 μG KCN ON THE OXYGEN SATURATION, EXPRESSED AS D560/D505 OF ARTERIAL AND VENOUS BLOOD OF FEMALE CBA/RIJ MICE ABOUT 20 G

(Urethane 1 g/kg (0·5 g intraperitoneally, 0·5 g subcutaneously.))

	Arterial blood			
Before KCN (*A*)	After 100 μg KCN (*B*)			Difference (*B*–*A*)
	3 min	5 min	10 min	
1·82	1·82			0
1·78	1·78			0
1·80	1·80			0
1·82		1·82		0
1·81		1·80		−0·01
1·81		1·85		−0·04
1·79		1·82		+0·03
1·82			1·83	0·01
1·83			1·83	0
	Venous blood			
2·35	2·21			−0·14
2·18	2·00			−0·18
2·08	2·05			−0·03
2·19		2·00		−0·19
2·22		2·01		−0·21
2·11		2·01		−0·10
2·24			2·00	−0·24
2·20			2·01	−0·19

TABLE 7. THE EFFECT OF KCN ON THE BLOOD PRESSURE OF UNANAESTHETIZED FEMALE CBA/RIJ MICE OF ABOUT 20 G

(Average deviations from the pre-injection values are given in mm Hg.)

KCN (μg)	Number of mice	Average change in blood pressure after KCN (mm Hg)					
		1 min	2 min	5 min	10 min	20 min	40 min
100	10	−53	−25	−23	−18	−12	−4
75	7	−46	−23	−20	−17	− 6	− 5
50	6	−48	− 7	− 3	− 5	0	–
25	8	− 4	+ 8	+14	+ 4	− 2	–

We were unable to determine the blood flow through the mouse spleen, but it was assumed that if KCN acted on the vasomotor centre the oxygen tension in the brain would not be reduced since the brain vessels are considered to be non-reactive to a vasomotor stimulus.

The effect of KCN *on the oxygen tension in the brain*

We felt that the implantation of an electrode in the brain simultaneously with an electrode in the spleen was too heavy a strain for a mouse. Therefore the oxygen tension in the brain was recorded in a number of separate experiments. The results of sixteen experiments with 100 μg KCN are presented in Table 8.

Although the results show a large degree of variation the general trend is clearly that a very short initial fall is followed by a substantial rise of much longer duration.

The initial decrease is probably caused by the large initial fall in blood pressure whereas the secondary rise corresponds in its duration to the reduction of the oxygen tension found in the spleen.

TABLE 8. THE EFFECT OF 100 μG KCN ON THE OXYGEN TENSION IN THE BRAIN OF UNANAESTHETIZED FEMALE CBA/RIJ MICE OF ABOUT 20 G

Exp. no.	Initial fall (%)	Duration (min)	Secondary rise (%)	Duration (min)
1	69	4	53	18
2	88	2	11	20
3	32	4	150	30
4	19	2	67	52
5	33	6	0	—
6	17	2	56	20
7	32	6	8	21
8	20	2	350	27
9	21	2	125	30
10	17	2	83	34
11	27	2	73	35
12	0	—	92	34
13	86	3	93	35
14	10	3	60	20
15	0	—	138	26
16	10	2	200	40
average ± s.d.	29 ± 7	2·6 ± 0·43	97 ± 22	28 ± 5

In a few rats of about 200 g the oxygen tension in the spleen and brain were measured simultaneously before and after 500 μg of KCN. In these experiments the similarity in time course of the two effects was clearly demonstrated.

DISCUSSION

The experiments described in this paper show that doses of KCN which protect mice against a lethal dose of total body irradiation reduce the oxygen tension in the spleen and bone marrow of unanaesthetized mice under conditions comparable to the irradiation experiments. In a previous paper[1] experiments were described in which the reduction of the oxygen tension in the spleen caused by injection of a number of protective amines as well as by a lowering of the oxygen content of the inspired air, was compared with the protection against radiation afforded by these experimental procedures. These experiments strongly indicate that for the compounds studied the protection against radiation is mediated by a reduction of the oxygen tension in a critical blood-forming organ.

When the results obtained with KCN are compared with these previous experiments it appears that the values for the 100 μg dose fit into the curve showing the relation

between percentage protection and percentage reduction of oxygen tension in the spleen. However, at lower doses of KCN the protection is somewhat less than might be expected from the reduction of the oxygen tension in the spleen. A possible explanation of this discrepancy may be offered by the fact that the duration of the effect was not taken into account in the construction of the curve showing the relation between protection and reduction of oxygen tension. Especially after 50 μg of KCN the effect on the spleen is over before the end of the period of irradiation. On the other hand a radiomimetic effect of cyanide has been described in a number of biological objects. For instance Read[7] described an inhibition of growth, while Lilly and Thoday[8] described an inhibition of mitosis and an increase in the number of chromosome aberrations in *Vicia faba* roots. Kihlman[9] also investigating *Vicia faba* roots described the formation of chromosome breakages by KCN and Kihlman *et al.*[10] showed that the number of chromosome breakages caused by X-rays was increased by KCN under anaerobic conditions. Wagner *et al.*[11] showed that cyanide increases the mutation frequency in Neurospora crassa, whereas Sobels[12] found that cyanide increases the number of sex-linked lethal mutations caused by X-rays in *Drosophila*. A similar effect in the mouse superimposed on the protection caused by hypoxia might tend to decrease the protective capacity.

Whatever may be the explanation of this slight discrepancy there is no reason to assume that the protection afforded by KCN to mice is due to a mechanism other than reduction of the oxygen tension in the spleen and bone marrow. Evidently these experiments give no indication as to the way in which this hypoxia causes protection at the cellular level.

The reduction of the oxygen tension in the spleen is somewhat unexpected as it is known that due to the inhibition of cytochrome oxidase the oxygen uptake in the tissues is lowered and as a consequence the oxygen saturation of the venous blood is increased. The experiments reported in the present paper show accordingly that at the protective doses used, the total oxygen consumption of the animal is greatly reduced and the saturation of the venous blood is increased while the arterial blood shows a normal saturation.

As it was shown that the release of oxygen from the haemoglobin is not affected by KCN,[13] the hypoxia in the spleen could only be caused by a substantial impairment of the blood supply to this organ. According to Pilcher and Sollmann[14] an intense stimulation of the vasomotor centre is seen after KCN. Although we were unable to demonstrate vasoconstriction in the different organs directly, we could show that the oxygen tension in the brain increased considerably concurrent with the decrease in the spleen. This is in accordance with the view that KCN stimulates the vasomotor centre in a similar way as it is stimulated during asphyxia, causing vasoconstriction in all organs which are sensitive to a vasomotor stimulus (such as the spleen) but leaving a number of organs (such as the brain) unaffected.

In the spleen the hypoxia caused by vasoconstriction then overcompensates the reduced oxygen uptake due to cytochrome-oxidase inhibition whereas in the brain the cytochrome-oxidase inhibition, possibly combined with an increased blood flow, becomes apparent as a rise in oxygen tension.

The only experimental fact that does not fit into this picture of vasomotor stimulation, is the fall in blood pressure where a rise was to be expected. Possibly this fall

in blood pressure is caused by a vasodilatation in the mesenteric vessels as was observed by Bean and Sidky[15] on local application of cyanide. This vasodilatation then might reduce the venous return. On the other hand other possibilities such as vasodilitation in different parts of the body (e.g. the brain) or a direct action on the heart muscle cannot be excluded by the present experiments. Konstantinova and Grayevsky[16] found a reduction of the oxygen tension in the spleen not exceeding 25 per cent after 150 μg of KCN was injected subcutaneously. Possibly this smaller effect may be explained either by differences in the strains of mice used or by the fact that these authors injected the compound subcutaneously while we used the intraperitoneal route. In accordance with the slight reduction of the oxygen tension in the spleen they found no protection against a dose of 900 r of ^{60}Co γ-rays.

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