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POTASSIUM-INDUCED INCREASE IN OXYGEN CONSUMPTION OF BROWN ADIPOSE TISSUE FROM THE RAT

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SUMMARY

1. In brown adipose tissue, noradrenaline induces an increase in respiration and a depolarization of the cells. The effect of an increase in potassium concentration in a range known to depolarize the brown adipocytes was tested on the O_2 consumption.

2. Isolated interscapular brown adipose tissue from the rat was incubated in chambers that allowed O_2 consumption to be measured over prolonged periods.

3. 45–50 mM-KCl were found to induce a more than fourfold increase in O_2 consumption, which was stable, reversible and dependent upon the presence of calcium in the medium.

4. When rats were pre-treated with reserpine or 6-hydroxydopamine the KCl-induced increase in O_2 consumption was sharply reduced or entirely absent.

5. The effect of KCl was greatly inhibited by (−)-propranolol, but not by (+)-propranolol.

6. Moderate increases in O_2 consumption induced by low concentrations of potassium were potentiated by desipramine, a drug which is known to block the uptake of catecholamines by adrenergic nerve endings.

7. Surgical denervation caused a decrease in the catecholamine content of the tissue, but had no effect on the KCl response.

8. It is concluded that in brown adipose tissue, potassium stimulates O_2 consumption by causing a release of noradrenaline from nerve endings. This implies that surgical denervation as it is commonly performed on this tissue does not denervate the brown adipocytes but probably only the blood vessels.

INTRODUCTION

In numerous non-excitable tissues, hormones, neurotransmitters and other specific stimuli produce a change in membrane potential of the target cells. In endocrine pancreas, for example, insulin release promoted

by glucose is accompanied by depolarization of the β-cells (Dean & Matthews, 1970); a depolarization also occurs in exocrine pancreas when amylase release is induced by acetylcholine or pancreozymin (Matthews, Petersen & Williams, 1973). A generalized scheme has been presented (Rasmussen, 1970) in which it is suggested that the primary signal in effector cells is a membrane depolarization accompanied by adenylate cyclase activation. Several attempts have been made subsequently to induce a decrease in membrane potential by raising the external concentration of potassium in order to determine whether this non-specific stimulus can mimic the action of the more specific stimuli. Such experiments have been reported to be successful in virtually all exocrine and endocrine glands.

In the present study, the same attempt was made with brown adipose tissue. The specific stimulus in this case is noradrenaline and the key event triggered by it is heat production. Noradrenaline activates a membrane-bound adenylate cyclase causing the formation of cyclic-AMP which activates a triglyceride lipase; the oxidation of the lipids thus released accounts for the heat production (Reed & Fain, 1970). Membrane depolarization of the brown adipocytes takes place as soon as noradrenaline reaches the cells (Girardier, Seydoux & Clausen, 1968; Williams & Matthews, 1974). The purpose of this study was to determine the extent to which depolarization induced by the addition of KCl could mimic the effect of noradrenaline on O_2 consumption.

METHODS

Preparation

Male Sprague–Dawley rats weighing from 280 to 380 g were used. They were kept at 23° C with a 12 h illumination period per day and had free access to water and standard laboratory chow (Ref. A 04 UAR). After decapitation, two fragments 10–15 mm long, 0·5–1 mm thick, 5–12 mg wet weight of brown adipose tissue were rapidly dissected from one interscapular fat pad. The interval between killing the animal and introduction of both fragments into the chambers did not exceed 6 min. Unless otherwise specified, Krebs–Ringer bicarbonate buffer solution (Krebs) of the following composition (in mM) was used as a standard incubation medium: NaCl 116·8, $NaHCO_3$ 25, KCl 5·9, $MgSO_4$ 1·2, NaH_2PO_4 1·2, $CaCl_2$ 2·45, with streptomycin 50 mg/l., and glucose (5 mM) which was added as described below. The medium was gassed continuously with a mixture of 95 % O_2 and 5 % CO_2. The temperature was set at $30 \pm 0{\cdot}2$° C.

Measurements

Experiments were carried out in two identical chambers, as illustrated in Fig. 1. The experiments involved repeated O_2 uptake determinations, based on the principle of Vieira, Caplan & Essig (1972). The P_{O_2} of a bubble-free liquid phase enclosed in a thick-walled Lucite chamber was measured by a Clark O_2 electrode. At 10–15 min intervals, a pump partially exchanged the solution for a fresh one within 45–120 sec,

so that the largest P_{O_2} decreases during each period of O_2 uptake determination did not exceed 55 mmHg. The fluid within the chamber was vigorously mixed by means of a magnetic stirrer in order to prevent the formation of a thick unstirred layer around the tissue. This also provided a rapid circulation of the fluid over the surface of the electrodes. Readings of the rate of O_2 disappearance (dP_{O_2}/dt) were made during the time the pump was not working. The O_2 electrodes (Eschweiler Cat. no. Ea 1-AC) were connected to a polarographic circuit whose output voltage was directly proportional to P_{O_2} (Beckmann Inst. RM Dynograph, polarographic coupler type 9871).

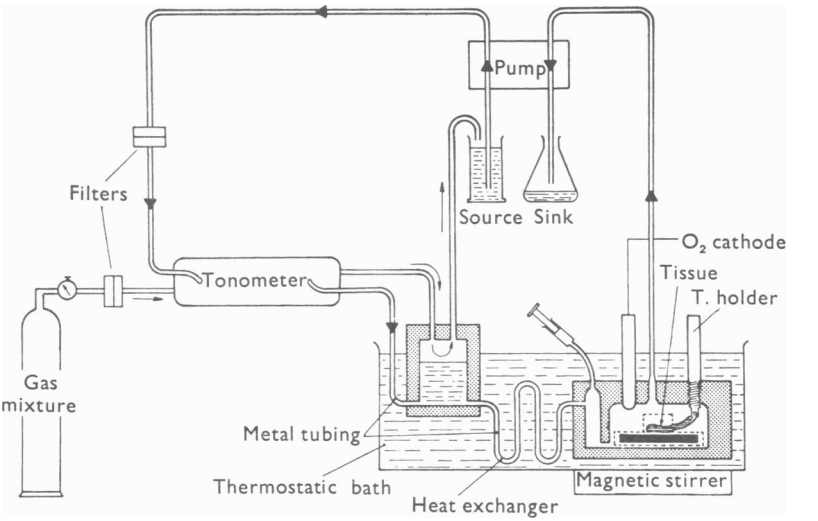

Fig. 1. Diagram of the experimental set-up. The O_2 cathode measures the P_{O_2} of the well-stirred liquid in the chamber, which contains the tissue sample. This chamber is intermittently perfused by means of the peristaltic pump with filtered, tonometered and thermostabilized medium. An expansion vessel compensates for small flow-rate differences between the two channels of the pump and prevents hydrostatic pressure changes upon introduction and removal of the preparation; during either of these procedures, the top of the chamber is exposed by lowering of the thermostatic bath.

Between experiments, the chambers were continuously exposed to liquid equilibrated with 95 % O_2, in order to keep the Lucite walls equilibrated with a high P_{O_2}. Rinsing solution followed by Krebs solution was pumped through the chambers for at least 1 h before each experiment to provide stable readings of P_{O_2}. Each tissue sample was then introduced with a tissue holder screwed into the chamber, a procedure which prevented large P_{O_2} decreases (< 50 mmHg). At the end of the experiment, tissue samples were removed and weighed while recording continued for 10 min in order to check for any significant O_2 consumption due to microbial contamination. In most of the experiments, there was no detectable decrease in P_{O_2} after the tissue was removed. The following procedure was adopted to avoid microbial contamination: the set-up was rinsed with a 1 % formaldehyde solution, millipore filters were placed on both gas and fluid channels, all the instru-

ments used for dissection as well as the syringes through which glucose was added to the medium as a pasteurized concentrated solution were sterilized. When O_2 consumption was detected in the absence of tissue, the experiment was discarded.

The electrodes were calibrated with gas mixtures containing 95 and 90 % O_2. The O_2 uptake rate ($\dot{M}_{O_2}$), in nmol. mg wet wt.$^{-1}$ h^{-1} was calculated according to the equation:

$$\dot{M}_{O_2} = \frac{S}{W} \frac{(P - P_{H_2O})\ (0{\cdot}95\text{–}0{\cdot}90)}{E - E'} \frac{\alpha . V . 10^6}{760 . 22{\cdot}4},$$

where S is the slope of the recorded plot (mV/h), W is the wet weight of the tissue (mg), P is the barometric pressure, P_{H_2O} is the water vapour pressure at 30° C, 0·95 and 0·90 are the O_2 fractions in the calibration gasses, E and E' are the output voltages when the electrode is exposed to 95 and 90 % O_2 mixtures, α is the solubility coefficient of O_2 at 30° C (0·02495 ml. O_2 s.t.p.d. per ml. Ringer solution per atm. O_2) and V is the volume of fluid in the chamber (3·5 ml.).

Results for $\dot{M}_{O_2}$, as well as for catecholamine content, are expressed as the mean ± S.D. of an observation.

Additions to the medium

Glucose, (−) −, (+) −, and (±)-propranolol (Imperial Chemical Industries Ltd), (−)-noradrenaline (Fluka) and desipramine (Ciba-Geigy) were added to the medium by means of 20 ml. motor-driven syringes (Dauerinfusions Gerät Perfusor, Braun Melsungen, Germany) connected to needles set at the inlet of each chamber.

Reserpine treatment, surgical and chemical denervation

Reserpine (Serpasil, Ciba-Geigy) 6 mg/kg was injected intraperitoneally 15 h before the experiments. The interscapular brown adipose tissue of nine rats weighing from 80 to 100 g was surgically denervated on one side by the method of Sidman & Fawcett (1954). This method involves cutting the nerves entering the brown fat pad at its ventromedial face. The opposite side was not touched and served as a control. The tissues from five animals were used 4 weeks later for O_2 uptake determinations and on four rats the catecholamine content was measured for both sides. 6-Hydroxydopamine hydrochloride (Fluka) was dissolved in distilled water containing 0·001 N-HCl and equilibrated with N_2. Two doses of 6-hydroxydopamine (100 mg/kg) were injected I.V. four days apart. Animals were used 24 h after the second injection for O_2 uptake determinations and catecholamine measurements.

Measurement of catecholamine content

Tissue pieces (275–466 mg wet weight) were homogenized in 0·4 N perchloric acid and purified by passage over a column of Amberlite CG 50 (potassium ion-form, pH 6·0). Noradrenaline was measured fluorimetrically by the trihydroxyindole method, as modified by Geissbühler (F. Geissbühler, to be published) in the column eluates. With this technique, the lowest detectable amount, as defined by the content of noradrenaline giving a fluorimetric signal twice that of the blank, was estimated to be 84 ng/g wet wt.

RESULTS

(1) *Basal O_2 consumption and effects of the addition of KCl*

Fig. 2 shows the spontaneous evolution of the O_2 consumption (open circles): a sharp decrease in $\dot{M}_{O_2}$ was observed during the first few minutes after introduction of the tissue sample into the chamber, followed by a

less marked decrease during the next 90–120 min. No large spontaneous fluctuations were observed for the following 4 h. Mean basal $\dot{M}_{O_2}$ as determined after the first 2 h of incubation was $43{\cdot}5 \pm 12{\cdot}5$ nmol. mg wet wt.$^{-1}$h^{-1} ($n = 61$).

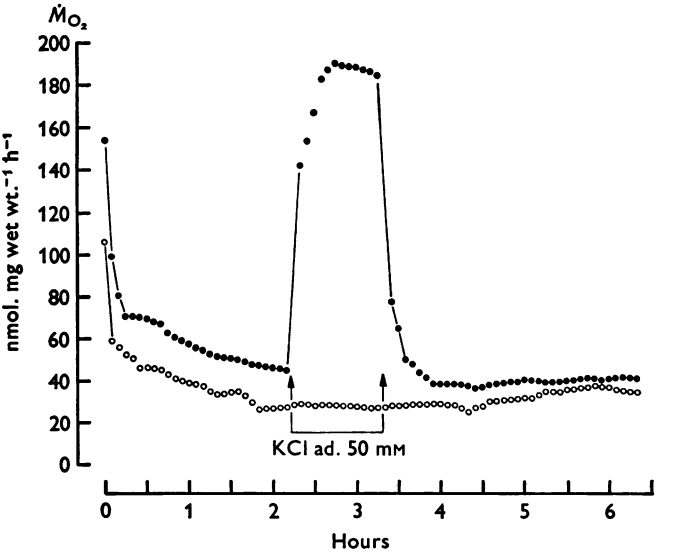

Fig. 2. O_2 uptake rate as a function of time of two brown adipose tissue samples from the same animal. The open circles represent the spontaneous O_2 consumption of the control sample in the standard medium; no large fluctuations are seen after the first 2 h of the experiment. The filled circles show the reversible effect of 60 min of exposure to a KCl-enriched medium (+44·1 mM).

To determine the effects of an increase in potassium concentration, 44·1 mM-KCl was added to the medium; this amount had been shown to depolarize the cells by 25–30 mV (Girardier *et al.* 1968). The filled circles (Fig. 2) show that KCl addition induced an increase in respiration, which was completely reversible when the standard medium was reintroduced. The mean of maximum $\dot{M}_{O_2}$ values obtained during the KCl stimulations was $188{\cdot}1 \pm 45{\cdot}4$ ($n = 19$), with KCl concentrations ranging from 45 to 50 mM.

(2) *Effects of KCl on tissues from reserpine-treated animals*

The possibility that KCl could depolarize the nerve endings included in the preparation and induce noradrenaline release was then considered. It has been clearly demonstrated that nerve varicosities containing small dense-core vesicles lie in close contact with the adipocytes (Bargmann, Hehn & Lindner, 1968; Ochi, Konishi & Yoshikawa, 1969) and that these

varicosities display the histochemical fluorescence properties typical of catecholamine-containing structures (Wirsén & Hamberger, 1967). This possibility was tested by injecting four rats with reserpine (see Methods), a drug which is known to deplete the nerve endings of their catecholamine content. Basal $\dot{M}_{O_2}$ ($38{\cdot}0 \pm 9{\cdot}6$, $n = 4$) was not modified by this treatment, but in all four experiments the addition of KCl did not cause an increase in respiration (Fig. 3). Furthermore, the mean $\dot{M}_{O_2}$ value following addition of noradrenaline (10^{-6} M) was $205{\cdot}5 \pm 32{\cdot}7$ ($n = 4$), which is not significantly different from the value obtained with samples taken from non-treated animals, i.e. $218{\cdot}2 \pm 48{\cdot}4$ ($n = 5$).

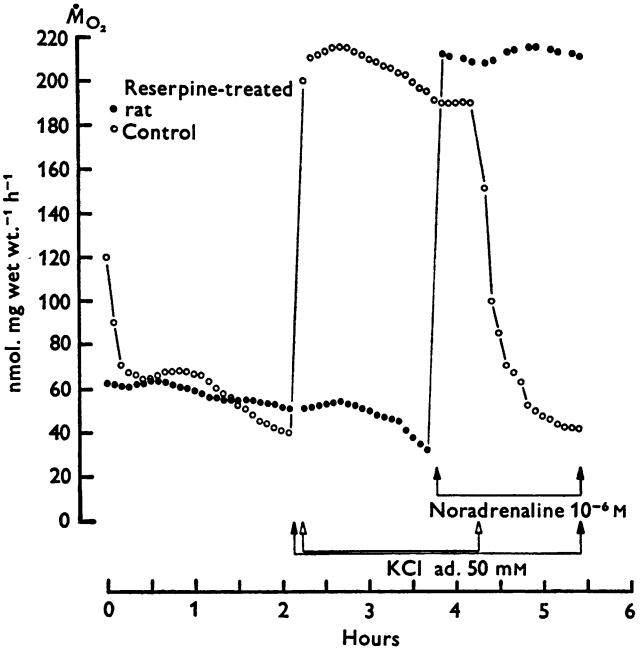

Fig. 3. Experiment with a sample taken from a reserpine-treated animal (filled circles) and a control sample (open circles). The former shows no response upon exposure to the 50 mM-KCl medium, but an increase in O_2 consumption upon the addition of noradrenaline. High initial $\dot{M}_{O_2}$ values are noticeably absent. The latter (open circles) shows the normal reversible response to KCl.

(3) *Influence of calcium concentration on the KCl response*

Since reserpine can have several side effects not related to catecholamine depletion (see, for example, Gillis & Lewis, 1956), further experiments were performed to test the hypothesis that the KCl effect was caused by a release of endogenous catecholamines. Since the requirement

for calcium and the antagonistic effect of magnesium are known to be important characteristics of the neurotransmitter release process (del Castillo & Katz, 1954), the effects on the KCl response of a lowered calcium concentration (10^{-6} M) and an increased magnesium concentration (10^{-2} M) were studied. This produced a rapid and complete suppression of the KCl response (Fig. 4). Under these conditions the tissue remained responsive to the addition of noradrenaline which caused an increase in $\dot{M}_{O_2}$ (mean $\dot{M}_{O_2} = 210{\cdot}6 \pm 15$, $n = 5$). In four experiments the KCl response was suppressed by lowering the calcium concentration to 10^{-5} M, without changing the magnesium concentration, although in this case the effects of the KCl stimulation disappeared more slowly.

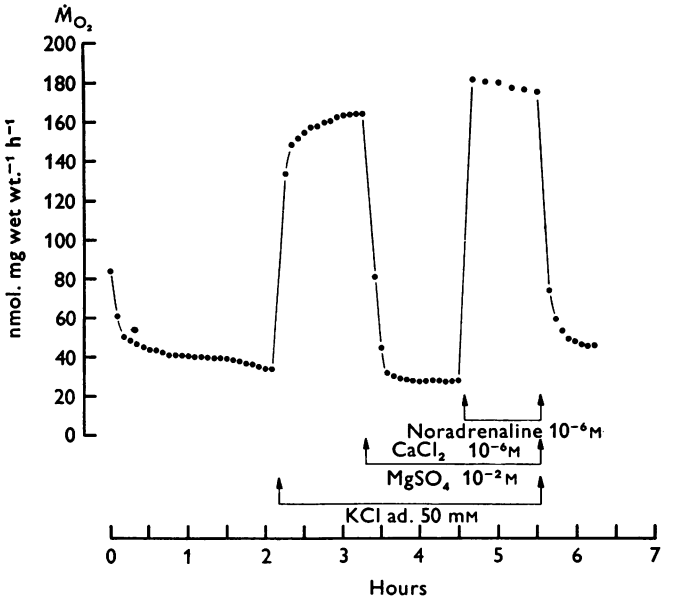

Fig. 4. The effects of a low calcium (10^{-6} M), high magnesium (10^{-2} M) medium on the KCl response. It can be seen that this response is completely suppressed, while in contrast, that to noradrenaline is unaffected.

(4) *Effects of (−)- and (+)-propranolol on the KCl response*

Propranolol has been shown to efficiently prevent the β-effects of catecholamines in many tissues including brown adipose tissue (Heim & Hull, 1966). Specificity of a β-adrenergic block can be assessed by using the criteria of both low dose and stereospecificity (Nickerson, 1970).

The KCl response was found to be completely suppressed by (±)-propranolol at 10^{-6} M ($n = 7$). At 10^{-7} M it was possible to distinguish between

the effects of the two isomers (Fig. 5). It was found that the effect of propranolol was not reversible for at least 1 h ($n = 2$).

(5) *Effects of desipramine*

Noradrenergic nerve endings have the distinctive ability to take up part of the neurotransmitter they secrete, a process which plays an important role in terminating the action of noradrenaline (Molinoff & Axelrod, 1971). Desmethylimipramine (desipramine) is one of the most

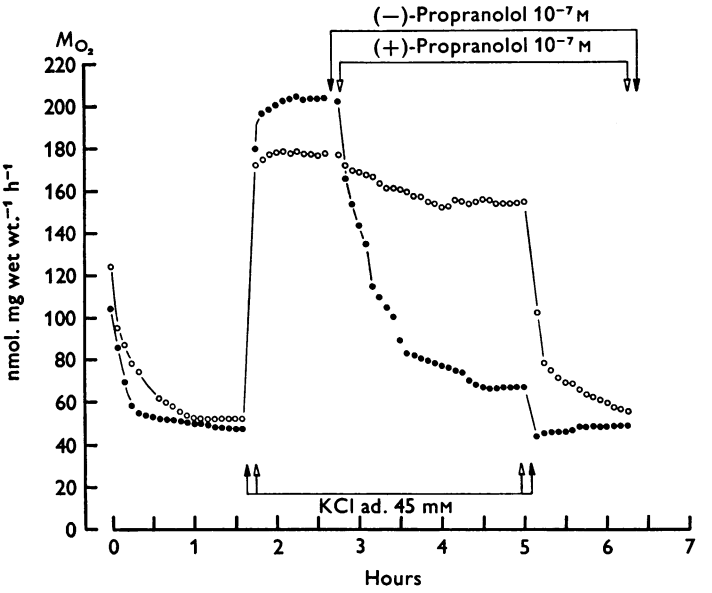

Fig. 5. Comparison of the effects of (−)-propranolol (filled circles) and (+)-propranolol (open circles) on the KCl response in two samples from the same animal. At the concentration used, 10^{-7} M, the inhibitory effect of the (−)-isomer is obviously much greater than that of the (+)-isomer.

potent blockers of this reuptake (Iversen, 1974). It was found that moderate KCl responses (i.e. 20–120 % increases in basal O_2 consumption brought about by the addition of 19·1–29·1 mM-KCl to the standard medium) were enhanced by the addition of desipramine (10^{-6} M) (Fig. 6). The mean increase in $\dot{M}_{O_2}$ caused by this drug was $69{\cdot}8 \pm 20{\cdot}3$ ($n = 6$).

Desipramine had no effect on basal O_2 consumption, except when it was added at the beginning of the experiments, in which case it clearly prolonged the initial phase of high O_2 consumption ($n = 3$). Even after 90 min, the longest period tested, its potentiating effect on KCl responses was not reversible.

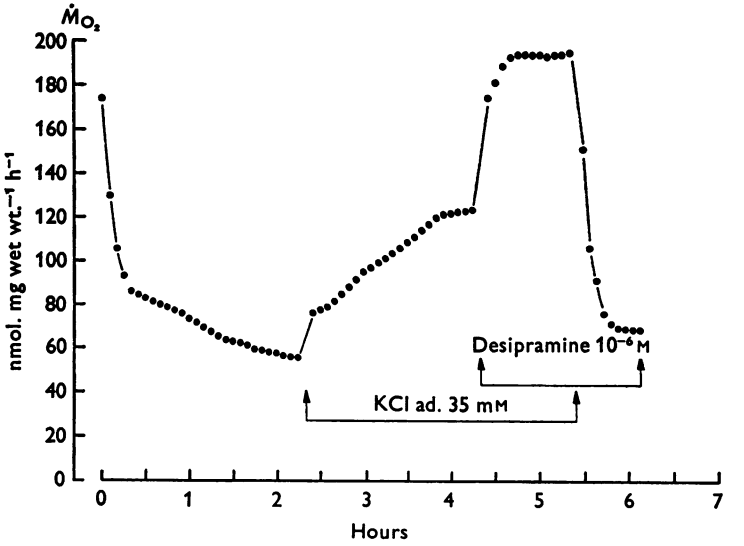

Fig. 6. Potentiation by desipramine of the moderate increase in O_2 consumption caused by an addition of 29·1 mM-KCl to the normal medium. Desipramine alone has no effect on basal O_2 consumption when added after the first 30 min of experiment.

(6) *Effects of surgical and chemical denervations*

In view of these results, which all argue for attributing the KCl effect to a release of noradrenaline from nerve endings, surgical denervation of the tissue was performed (see Methods) and catecholamine content was measured; this was found to be $892 \pm 236{\cdot}4$ ($n = 4$) ng/g wet wt. for the control side and $355 \pm 223{\cdot}5$ ($n = 4$) for the denervated side. O_2 consumption experiments were conducted with a sample from the denervated side in one chamber and a sample from the contralateral control side in the other. The paired t test (control side *vs.* denervated side) showed no significant differences in the amplitude of the KCl response on the two sides ($P > 0{\cdot}1$, $n = 5$). This result is in agreement with those of Derry & Daniel (1970) who concluded from their fluorescence data that the nerves cut by the Sidman & Fawcett method innervate the blood vessels of the brown adipose tissue but not the adipocytes (see Discussion). In order to eliminate both the innervation of blood vessels and that of the adipocytes, four rats were chemically denervated with 6-hydroxydopamine, a drug that has been shown to destroy the sympathetic nerve endings selectively (Thoenen & Tranzer, 1968). In these animals the catecholamine content of brown adipose tissue was reduced to below the lowest detectable level (< 84 ng/g wet wt., $n = 4$), and the KCl response was slight and transitory (Fig. 7). It is also shown that the response to noradrenaline

was still present; however, its amplitude very markedly decreased with time. No satisfactory explanation has been found to account for this unstable noradrenaline response, which occurred only in the experiments with 6-hydroxydopamine-treated rats. For these chemically denervated samples, mean basal $\dot{M}_{O_2}$ value was $44{\cdot}2 \pm 10{\cdot}4$ ($n = 4$) and the highest mean $\dot{M}_{O_2}$ values were for KCl stimulation $57{\cdot}8 \pm 20{\cdot}3$ ($n = 4$) and for noradrenaline stimulation $238{\cdot}6 \pm 50{\cdot}0$ ($n = 4$).

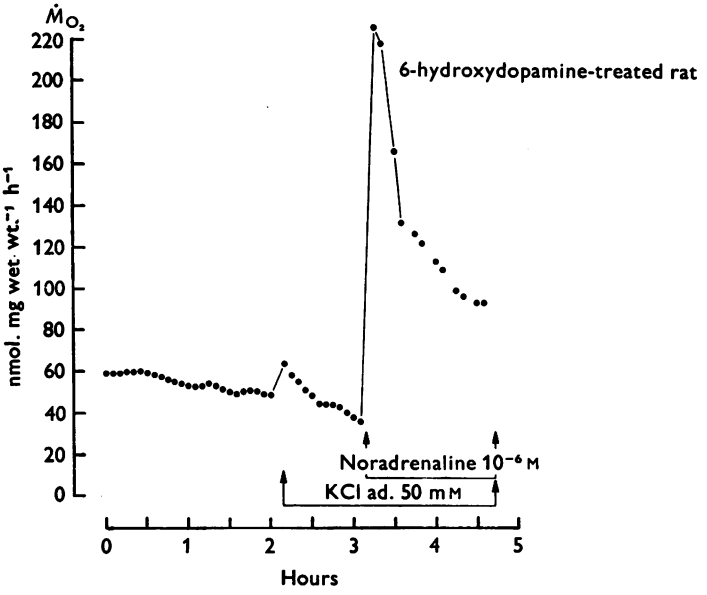

Fig. 7. Experiment performed on a sample taken from a 6-hydroxydopamine-treated rat. The KCl response is almost completely suppressed; the amplitude of noradrenaline response is normal during the first minutes after its addition and then decreases sharply with time.

Fig. 7 also shows that the initial phase of high O_2 consumption was absent. In all the tissue samples taken from reserpine or 6-hydroxydopamine-treated rats, this phase was either absent or sharply reduced in amplitude (also illustrated in Fig. 3, filled circles). It would seem, therefore, that the initial phase of high oxygen consumption may be due to a release of catecholamines from nerve endings which were damaged during dissection of the tissue. The enhancing effect of desipramine on O_2 consumption during this phase would seem to support this interpretation.

DISCUSSION

The basal $\dot{M}_{O_2}$ values reported in this study, when corrected for temperature differences ($Q_{10} = 2$), fall within the range of values obtained for brown adipose tissue of the rat by other investigators (Joel, 1970; Yoshimura & Hiroshige, 1970; Drahota & Vízek, 1971).

The stimulatory effect of potassium on the O_2 consumption in this tissue has already been reported (Yoshimura & Hiroshige, 1970; Drahota & Vízek, 1971); it has also been noted that calcium is required for this effect and that magnesium antagonizes it (Yoshimura & Hiroshige, 1970). These results were reproduced in the present study and it is suggested that they can be most plausibly explained by a release of noradrenaline from the nerve terminals. The following observations support this hypothesis: the KCl effect is suppressed when the rat is given a prior treatment with reserpine and sharply reduced by treatment with 6-hydroxydopamine; it is strongly diminished by (−)-propranolol and is potentiated by desipramine. The fact that surgical denervation does not suppress the KCl response is not inconsistent with this hypothesis if it is accepted that the denervation produced by this procedure is restricted to the blood vessels, which would account for the decrease in the catecholamine content of the whole tissue. As shown by histofluorescence, blood vessels are more densely innervated with adrenergic nerve terminals than adipocytes, whose innervation is in fact sparse and was even thought for several years to be non-existent (Wirsén, 1964). Furthermore, this interpretation is perfectly consistent with the results of Derry & Daniel (1970); their fluorescence data showed that after surgical denervation, the innervation of the adipocytes was preserved and that of the blood vessels disappeared; they suggested that the nerves terminating on the adipocytes run close to blood vessels and are, therefore, not cut by the surgical procedure used. It can thus be inferred that the KCl effect is due to the release of noradrenaline from nerve endings that lie in close contact with the adipocytes.

In view of this finding, experiments were performed to determine whether 50 mM-KCl solution would depolarize the brown adipocytes of rats treated with reserpine. The same electrophysiological set-up previously described (Girardier *et al.* 1968) was used. It was found that 50 mM-KCl still induced a depolarization of the same amplitude as that seen in untreated rats, i.e. 25–30 mV. This indicates that external potassium has a direct depolarizing influence on the membrane potential which is not mediated by the release of noradrenaline from the nerve endings. It can, therefore, be concluded that a KCl-induced depolarization greater than 25 mV cannot directly modify O_2 consumption; it is suggested that the

increase in respiration is an indirect effect and results from a release of noradrenaline from the nerve endings of the adipocytes.

More generally, this study provides further evidence that in small pieces of tissue, nerve endings retain their physiological characteristics for several hours, and supports the hypothesis of Schramm (1968) and Argent, Case & Scratcherd (1971) suggesting an indirect action of KCl on many tissues. It was clearly shown in both of these studies that KCl-induced amylase secretion is in fact mediated by a release of acetylcholine in exocrine pancreas (Argent *et al.* 1971) and by a release of catecholamines in salivary glands (Schramm, 1968). Similar studies remain to be done for nearly all other secretory glands as it is only in the adrenal medulla and in the nervous tissue that a direct KCl stimulation of secretion has been established.

It would be of interest to do a study of this kind on the endocrine pancreas where there is a double adrenergic and cholinergic innervation, which persists even after isolation of Langerhans islets by collagenase (Orci, Perrelet, Ravazzola, Malaisse-Lagae & Renold, 1973). Although several reports indicate an insulin release by KCl, only one (Hales & Milner, 1968) mentions that atropine sulphate (0·1 mg/l.) does not suppress the release induced by 60 mM-KCl. It is possible that this dose was not sufficient to suppress an effect mediated by a release of acetylcholine. Argent *et al.* for example (Argent *et al.* 1971) used atropine 10 mg/l. with 50 mM-KCl and Matthews *et al.* (Matthews *et al.* 1973) used 2 mg/l. with 47 mM-KCl to block the effects of acetylcholine in exocrine pancreas.

It has been suggested by Argent *et al.* (1971) that only secretory tissues of neural origin are directly stimulated by potassium; we would extend this suggestion and propose that in all non-excitable tissues, with the exception of adrenal medulla (a tissue of neural origin), a change in membrane potential caused by potassium is not able to trigger directly the specific response of the tissue.

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