AN. INST. BIOL. UNIV. NAL. AUTÓN. MÉXICO 41, SER. BIOL. EXP. (1): 17-24, 1 fig., 2 tablas (1970)

ON THE MECHANISM OF THE ANOREXIGENIC EFFECT OF ADRENALINE AND AMPHETAMINE *

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ABSTRACT

Intraperitoneal (i.p.) adrenaline (*A*) elicited: a strong anorexia and a substantial reduction in spontaneous motor activity which increased with the dose; a mild increase or no effect in oxygen consumption (QO_2); negligible changes in rectal temperature (*RT*); a marked hyperglycemia; and a significant reduction in liver glycogen (LG) that could easily account for the hyperglycemia quantitatively. On the other hand, the isoanorexigenic close of amphetamine (*Am*) *elicited*: a great increase in erratic spontaneous activity; a large increase in QO_2 and *RT*, no hyperglycemia, but a similar decrease in *LG*. Therefore, both substances caused the same increase in the rate of glucose liberation by the liver, but in the case of *Am*, the glucose was consumed by the marked motor activity, resulting in little hyperglycemia. Nevertheless, if the hepatic glucoreceptors are sensitive to the rate of glucose transport through the cell membrane, they could be the main cause of the anorexia produced by both *A* and *Am*. The hypothetical sequence of events would be, for *A*: a primary glycogenolytic effect causing satiation, which in turn causes decrease in activity; this masks or even reverses the primary calorigenic effects of *A*. No activation of the central nervous system (*CNS*) is produced, because most *A* is destroyed in the liver and the small amount that reaches the general circulation does not traverse the bloodbrain barrier (*BBB*). In the case of *Am*: there would be no primary glycogenolytic effects. Most of the substance would reach the general circulation, and would easily traverse the *BBB*, stimulate the *CNS* and produce the increase in activity, with a concomitant increase in sympathetic activity that causes an hepatic glycogenolysis. This latter is monitored by the hepatic glucoreceptors and produces anorexia. That the increase in activity is related to the other changes is shown by the lack of effect, of *Am* on QO_2 and *RT* in anesthetized animals.

RESUMEN

La adrenalina (*A*) intraperitoneal produjo: una fuerte anorexia y una disminución marcada de la actividad motora espontánea; un pequeño o nulo aumento en el consumo de oxígeno (QO_2); cambios pequeños en la temperatura rectal (*TR*); una marcada hiperglucemia; una reducción significativa del glucógeno hepático (*GH*) suficiente para explicar la hiperglicemia. Por otro lado, la dosis isoanorexigénica de anfetamina (*An*) produjo: un gran aumento de la actividad motora espontánea; un marcado aumento del QO_2 y de la *TR*; una reducción del *GH* similar a la producida por la adrenalina, pero sin ninguna hiperglucemia. Por lo tanto, ambas substancias produjeron el mismo aumento en la cantidad de glucosa liberada por el hígado, pero en el caso de la *An*, esta glucosa es rápidamente

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consumida debido a la gran actividad muscular, por lo que no se produce hiperglicemia.
Sin embargo, si los glucorreceptores hepáticos son sensibles a la cantidad de glucosa transportada a través de la membrana, podrían ser la causa que origina la anorexia tanto en el caso de la *A*, como en el de la *An*.
La secuencia hipotética de reacciones sería, para *A*; una glucogenolisis primaria que reduce las "señales de hambre" de los glucorreceptores hepáticos; la sensación de saciedad sería la causa de la tranquilización manifestada por la reducción de la actividad motora. No habría activación del sistema nerviso central (*SNC*) porque la casi totalidad de la *A* es destuida por el hígado y la poca que logra pasar a la circulación general no penetra la barrera hematoencefálica (*BHE*). En el caso de la *An*: no habría actividad glucogenolítica primaria. La mayor parte de la substancia llegaría a la circulación general, porque no es destruida por las enzimas hepáticas y una cantidad importante atravesaría la *BHE*, porque la *An* es menos polar que la *A*. La acción de la *An* sobre el *SNC* sería el aumento marcado de la actividad motora que a su vez produciría los aumentos de QO_2 y *TR*, y una activación de los nervios simpáticos del hígado, lo cual produciría glucogenolisis. Esta última actuaría sobre los glucorreceptores hepáticos y produciría la anorexia. La idea de que el aumento de actividad motora es la causa de los aumentos de QO_2 y *TR* recibió un fuerte apoyo en el hecho de que estos cambios desaparecieron cuando se eliminó el cambio de actividad motora anestesiando al animal.

INTRODUCTION

A highly significant inverse correlation was found between the changes in food intake and the changes in liver sugar concentration observed after the intraperitoneal administration of adrenaline, nor-adrenaline, glucose and insulin (Russek and Stevenson, in press). This further supported the hypothesis that hepatic glucoreceptors play an important role in the regulation of food-intake (Russek, 1963; Russek, 1967; Russek *et al.*, 1968a). On the other hand, amphetamine was found to produce a strong anorexia with a small increase in liver glucose, smaller than that predicted by the correlation obtained with the other substances mentioned above (Russek and Stevenson, in press). Nevertheless, amphetamine produced the same glycogenolysis as the isoanorexigenic dose of adrenaline, and the anorexia induced by both substances decreased when the glycogen reserves were diminished by a prolonged feeding with a carbohydrate free diet. This suggested that the anorexia produced by amphetamine was related to its glycogenolytic effect, which led to the question of why only adrenaline produced a marked hyperglycemia.

As the rats appeared very "excited" after amphetamine, it was thought that the glucose liberated by the liver could be consumed at the same rate in the muscles, which would explain the lack of hyperglycemia in spite of the glycogenolysis observed. To study this possibility, the effects of amphetamine, adrenaline and noradrenaline on spontaneous activity, oxygen consumption and rectal temperature were measured. Now, the changes, in oxygen consumption and rectal temperature, could be either secundary to the changes in muscular activity, or a primary effect of the substances, or both. To provide some evidence on this matter, the effects of adrenaline, noradrenaline and amphetamine on these parameters were determined in rats where muscular activity was abolished by anesthesia.

MATERIAL AND METHODS

Sixteen adult male Wistar rats, weighing 250-350 g were used in the study of muscular activity, oxygen consumption and rectal temperature. These animals were housed in individual cages, in a room lighted from 8 a.m. to 8 p.m., kept at a temperature of 22 ± 1°C. They were fed for 1 hour daily, a high-carbohydrate diet (70% sucrose, 21% casein, 4.5% corn oil, by weight, with adequate vitamins and minerals), with water available *ad libitum*. All the experiments were carried 1 hour prior to the feeding hour.

1. *Spontaneous activity*. The effects of amphetamine (1.0 mg/kg and 2.0 mg/kg) adrenaline (0.1 and 0.15 mg/kg), noradrenaline (0.1 mg/kg and 0.15 mg/kg) and isotonic saline on the spontaneous activity of the rats were determined using a "Jiggle" platform, with a piezoelectric pickup, on which the housing cage of the rat was located. A direct recording as well as the integrated mean of this activity was obtained with a Grass polygraph. After a control period of 30 min each animal was injected with one of the above mentioned solutions and reintroduced for another 30 min. The surface under the integrated curve was measured for sample periods of 5 min taken immediately before and 25 min after the injection.

2. *Oxygen consumption*. The method described by Ferguson and Sellers (1949) was used in these determinations. The rats were placed individually in each of four air tight cylindrical lucite tanks which were submerged in a thermostatically regulated water bath (28°C). The animals were allowed to breathe 100% oxygen warmed to the bath temperature. The CO_2 and water vapour were absorbed by barium hydroxide lime and the amount of oxygen consumed was measured at 10 min intervals by water displacement of the gas in a graduated cylinder, submerged in the same bath. After a period of 30 min during which a basal rate was obtained, the rats were removed, injected i. p. with one of the following substances, and re-introduced for another half hour period: adrenaline (0.15 mg/kg), noradrenaline (0.15 mg/kg), amphetamine (1.0 mg/kg and 2.0 mg/kg) and isotonic saline. A minimum interval of 2 days elapsed between consecutive injections to the same rat. In each experiment there was one rat injected with each of the four substances. The same substances were also administered to rats anesthetized with pentobarbital sodium (0.5 mg/kg) and oxygen consumption was determined as described above. All measurements were made 1 hr prior to the daily feeding.

3. *Temperature determinations*. Eighteen non-anesthetized rats and 24 anesthetized with barbitone (300 mg/kg) were used in this experiment. This anesthetic produces a more prolonged and constant anesthesia than pentobarbital, and provides a more stable body temperature base line. In the non-anesthetized rats the rectal temperature was measured after the thermistor (YSI Nº 402) had been in place (4 cm depth) for 1 min. Once a basal temperature was determined, adrenaline (0.15 mg/kb), noradrenaline (0.15 mg/kg) or amphetamine (2.0 mg/kg) was injected i.p. and the temperature measured again after 30 and 90 min. In the anesthetized rats, the temperature of the rectum was continuously recorded with the same rectal probe at the same depth as in the non-anesthetized rats. The animals were kept in a "Precision" incubator at an air temperature of 30°C. Injections were made only after the temperature had been stable for at least 15 min.

RESULTS

1. *Spontaneous activity.* The rats injected with amphetamine did not look calm and relaxed, as those injected whith adrenaline and noradrenaline, but exhibited a continous "tremor". The results obtained in the "jiggle cage" showed quite clearly a reduction in activity after adrenaline and an increase in general muscular activity after amphetamine (Table I). Even though it appears that the smaller dose of amphetamine produced more effect than the larger, the difference between them was not significant, while the difference between either dose and the control was highly significant. On the other hand, neither dose of noradrenaline produced any significant effect.

TABLE I

EFFECT OF I.P. ADRENALINE, NORADRENALINE AND AMPHETAMINE ON THE SPONTANEOUS ACTIVITY (JIGGLE CAGE) OF RATS

Substance (mg/kg)	Mean spontaneous activity (Area of integrated record in cm^2/min) Control	25' after Inj.	% of Control	N° of Rats
Isotonic saline	4.5	3.5	78	4
Noradrenaline (0.1)	1.9	1.7	89	5
Noradrenaline (0.15)	2.8	2.1	75	8
Adrenaline (0.1)	2.1	1.4	67	6
Adrenaline (0.15)	4.1	2.3	56	8
Amphetamine (1.0)	1.9	9.0	473	3
Amphetamine (2.0)	2.3	7.5	326	9

The data were obtained by integrating the activity in the 5 min period prior to injection, and the 5 min period, 25 min after the injection.

2. *Oxygen consumption* (QO_2). In table 2 it can be seen that no relation exists between the changes in QO_2 and the anorexigenic effects of the substances studied. Adrenaline and noradrenaline at the dose of 0.1 mg/kg produced the same increase in QO_2 but only adrenaline produced a significant decrease in food-intake. When the dose of adrenaline was increased to 0.15 mg/kg, its anorexigenic effect increased further, while its effect on QO_2 decreased to a non-significant level. When noradrenaline was increased to 0.15 mg/kg, it produced a mild but significant anorexia, while its effect on QO_2 became non-significant. On the other hand, amphetamine 2.0 mg/kg, which elicited aproximately the same effect on food intake as adrenaline 0.15 mg/kg, produced the largest increase in QO_2 (75% above the control). Half the dose of amphetamine (0.1 mg/kg), produced about 1/3 of the anorexigenic effect of the larger dose (29% decrease in food intake as compared to 87%) but increased QO_2 by 56%, which represented 3/4 of the effect of the larger dose. In the anesthetized

TABLE 2

EFFECT OF NORADRENALINE AND AMPHETAMINE ON THE OXYGEN CONSUMPTION (QO_2) OF RATS

Substance (mg/kg)	Condition	QO_2 (ml/kg $^3/_4$/min) 30′ control	30′ following Inj	% of Control	N	P (t-test)	Food-Intake (% of Control)
Saline	Nonanesthetized	11.53	11.46	99	16	NS	108 (+)
Noradrenaline (0.1)	Nonanesthetized	12.80	16.80 (*)	131	12	0.001	93 (*)
Noradrenaline (0.15)	Nonanesthetized	12.16	13.22	109	8	NS	66 (*)
Adrenaline (0.1)	Nonanesthetized	13.80	17.40 (*)	126	12	0.001	86 (*)
Adrenaline (0.15)	Nonanesthetized	12.87	14.82	115	8	NS	29 (+)
Amphetamine (1.0)	Nonanesthetized	10.91	17.00	156	12	0.001	71
Amphetamine (2.0)	Nonanesthetized	11.30	19.92	176	4	0.01	13 (+)
Amphetamine (2.0)	Anesthetized	7.59	8.80	116	8	NS	———

(*) From Russek, Mogenson and Stevenson (1967)

(+) From Russek, Stevenson and Mogenson (1968b)

SEM'S were not included for clarity sake. All were between 0.5 and 1.0.

animals, the effect of amphetamine (2.0 mg/kg) was only a small and non-significant increase.

3. *Rectal Temperature.* Amphetamine (2.0 mg/kg) in the non-anesthetized rats produced a substantial increase in rectal temperature which was highly significant (Fig. 1). This was in good agreement with the increase in QO_2 described before. Adrenaline and noradrinaline (0.15 mg/kg) produced no significant change during the first 30 min, which also agrees with the lack of effect on QO_2. On the other hand, adrenaline produced a significant decrease in temperature after 90 min, which might be due to the large decrease in activity induced by this dose of adrenaline as described above.

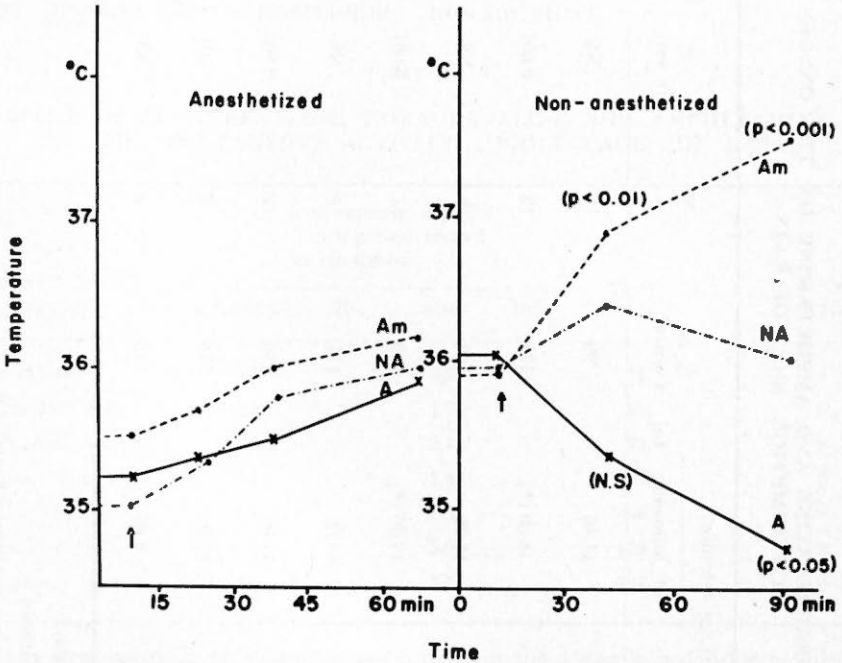

Fig. 1. The action of amphetamine 2.0 mg/kg (broken line), adrenaline 0.15 mg/kg (solid line) and noradrenaline 0.15 mg/kg (dash-and-dot), on the rectal temperature of anesthetized (left side) and non-anesthetized (right side) rats. Ordinates: temperature in °C. Abscissae: Time in minutes. The arrows mark the moment of injection. In parenthesis are the probabilities against the basal temperature. (N.S.-non-significant).

In the anesthetized rats adrenaline, noradrenaline and amphetamine produced only a small increase in rectal temperature very similar for the three substances. This suggested rather strongly that the change in rectal temperature observed in the non-anesthetized rats were related to the changes in activity, as both were eliminated by the anesthesia.

DISCUSSION

It is well known that adrenaline injected intravenously, produces marked tachycardia, hipertension, gastrointestinal relaxation, midriasis, hepatic and muscu-

lar glycogenolysis, lypolisis, and a strong arousal reaction. This latter is manifested by an increase in spontaneous orientation activity, so the animals move around nervously.

In the present paper, the adrenaline was injected intraperitoneally. As it went directly into liver, where it was almost completely destroyed, only negligible amounts of it reached the general circulation (Bloch, 1952; Russek, 1963). Therefore, one would expect that in this case the hepatic reactions would be stronger, while all the others would be much weaker or non-existent.

The results of this and previous papers (Russek *et al.*, 1967; Russek *et al.*, 1968b; Russek and Stevenson, in press) showed that the isoanorexigenic doses of adrenaline and amphetamine produced quite different effects on spontaneous activity, oxygen consumption (QO_2) rectal temperature and blood glucose. Adrenaline produced: a substantial decrease in activity, when the hungry rat was taken as control; a moderate increase in QO_2 at the low dose and no significant change at the high one; a small increase in rectal temperature at the low dose and a decrease at the high one; and a marked hyperglycemia (Russek *et al.*, 1967). The decrease in temperature was likely due to the fact that the anorexia produced by adrenaline was accompanied by a marked tranquilization. This was not a general impairment of the animal movements, as shown by the lack of effect of these doses of adrenaline on self-stimulation of locations that did not evoked any feeding, while decreasing the rate of self-stimulation that caused simultaneous feeding (Russek and Teitelbaum, 1968; Mogenson *et al.*, 1969). The lack of effect of the high dose of adrenaline on QO_2 and the decrease in temperature elicited by it are probably also caused by a greater decrease in activity, which masked the moderate direct calorigenic effect. This is corroborated by the fact that in the anesthetized rats, where spontaneous activity is eliminated, the high dose of adrenaline produces an increase in rectal temperature, instead of a decrease.

In spite of all the different or even opposite effects of the isoanorexigenic doses of adrenaline and amphetamine both produced a virtually identical reduction in liver glycogen (Russek and Stevenson, in press). Then, why in the case of adrenaline did the glycogenolysis resulted in a marked hyperglycemia and a large increase in liver reducing sugars (Russek and Stevenson, in press), while in the case of amphetamine there was no hyperglycemia and only a very small increase in liver sugars? The explanation is probably found in the different effects on activity and QO_2. In the case of adrenaline the liberation of liver glucose (aproximately 450 mg/kg/30 min) was accompanied by a small increase in QO_2, so a large part of this glucose remained in the blood. In the case of amphetamine, all the glucose liberated by the liver was utilized by the active muscles, which was reflected in the very high QO_2.

Now, how can the different effects of adrenaline and amphetamine be accounted for? An attractive speculation is the following: adrenaline, after being absorbed in the peritoneal cavity goes via the portal vein into the liver, where it produces a direct glycogenolytic effect, but at the same time most of it is destroyed by liver enzymes (monoaminooxidase and O-methyl-transferase). Therefore, only a small proportion passes beyond the liver and reaches the general circulation, which is shown by the negligible cardiovascular effects of adrenaline when injected i.p. as compared with the i.v. injection of the same dose

(Bloch, 1952; Rodríguez-Zendejas *et al.*, 1968). Moreover, from the small amount that reaches the arterial blood, an even smaller proportion traverses the blood-brain barrier (Weil-Malherbe *et al.*, 1959), so almost no central nervous system effects are obtained.

On the other hand, the arrival of amphetamine at the liver probably will not produce any direct glycogenolytic effect. As the enzymes that destroy adrenaline have much less or no effect on amphetamine, most of it reaches the general circulation, and being a less polar molecule than adrenaline, it traverses the blood-brain barrier and reaches the brain, where it elicites the great increase in motor activity observed. As a consequence of this increase in activity, or as a simultaneous central effect of amphetamine, a substantial increase in sympathetic activity is produced. Therefore noradrenaline liberated by the hepatic sympathetic nerves and an increase in circulating adrenaline, noradrenaline or glucagon would be the cause of the glycogenolysis. At the same time, the increase in glucose consumption by the active muscles would preclude the development of hyperglycemia.

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