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STUDY ON THE EFFECT OF 3-ACETYLPYRIDINE ON BLOOD GLUCOSE CONCENTRATION

MIGUEL PÉREZ DE LA MORA, RICARDO TAPIA and GUILLERMO H. MASSIEU

Departamento de Bioquímica, Instituto de Biología,
Universidad Nacional de México, México, D. F., México

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Abstract—It is known that the administration of 3-acetylpyridine (3-AP) to rats induces hyperglycemia. Experiments designed to study the mechanism of this action of 3-AP on blood glucose levels are reported.

The hyperglycemia produced by the administration of 3-AP to adult rats was accompanied by a decrease of hepatic and muscular glycogen. The hyperglycemic effect of 3-AP was significantly less in 8-day-old rats than in adult rats.

The simultaneous administration of the ganglionic blocking agent mecamylamine inhibited the hyperglycemic effect of 3-AP.

Small doses of 3-AP injected intracisternally produced hyperglycemia in short periods of time. The i.p. injection of the same dose of 3-AP did not exhibit such effect. Hyperglycemia was also produced by the intracisternal administration of the analogue of NAD, 3-AP-NAD, at a dose 100-fold less than the intracisternal dose of 3-AP which induced hyperglycemia.

The epinephrine content in the adrenal glands did not change significantly after the administration of a hyperglycemic dose of 3-AP.

It is concluded that the hyperglycemic effect of 3-AP is due to a release of catecholamines from the adrenal medulla and that this release is probably secondary to the lesions observed in brain after 3-AP administration. It is also concluded that 3-AP probably acts by forming the analogue 3-AP-NAD in brain.

IT IS KNOWN that 3-AP* induces extensive anatomical changes in the nervous tissue, especially in hypothalamus, hyppocampus, spinal and sympathetic ganglia, and adrenal medulla.[1, 2] In certain conditions 3-AP behaves as an antimetabolite of nicotinamide,[3–5] possibly by the formation of pyridine nucleotide analogues, in which the 3-AP substitutes for the nicotinamide fraction of the molecule, in a reaction catalyzed by NADase.[6] Kaplan *et al.*[7] have shown that these analogues can be reduced *in vitro* by several dehydrogenases, and more recent studies indicate that they can inhibit the activity of some pyridine nucleotide-dependent enzymes in brain.[8, 9]

Previous work from this laboratory has shown that the administration of 3-AP to rats induces a considerable hyperglycemia, which is prevented by previous adrenalectomy.[10] Since it is known that the mechanical stimulation of the hypothalamus produces hyperglycemia, it has been suggested that the lesions produced by 3-AP in this area can be related to its hyperglycemic action, possibly after the formation of 3-AP-NAD.[11] According to this idea, one of the consequences of the hypothalamic lesions would be a stimulation of the adrenal medulla to liberate epinephrine, which would be responsible for the observed hyperglycemia. Experimental approaches

* Abbreviations used: 3-AP, 3-acetylpyridine; 3-AP-NAD, 3-AP-analogue of NAD.

were made to test this hypothesis. The results of these experiments are presented in this work.

MATERIALS AND METHODS

Adult rats (local strain) weighing 250–300 g or 100–150 g and young rats (8 days old) weighing 10–14 g were used. Adult animals were fed with a commercial diet. 3-AP and 3-AP-NAD were obtained from the Nutritional Biochemical Corp., and 3-methylamine-isocamphane (mecamylamine) was also obtained commercially. 3-AP was injected into adult rats without previous dilution, but it was diluted with water to a suitable concentration when administered to young rats. 3-AP-NAD solution in water was used for injection of this compound. The drugs were administered i.p. and in some experiments 3-AP and 3-AP-NAD were injected intracisternally in adult animals. In the latter experiments the rats were fasted for 12 hr, anesthetized slightly with ether and the back of the neck was dissected to facilitate the injection of the compounds. Control rats were subjected to the same procedure except that saline solution was injected instead of 3-AP or 3-AP-NAD. The volume injected intracisternally was 10–20 μl.

In all cases the animals were decapitated and glucose was measured by the procedure of Nelson–Somogyi[12] in a sample of blood obtained at the moment of decapitation. Glycogen was determined in muscle and liver of control and treated nonfasted rats by the method of Good *et al.*,[13] using the Nelson–Somogyi method for the measurement of glucose in the hydrolysate.

Measurement of epinephrine in the adrenal glands. For the determination of epinephrine, the two adrenal glands were quickly removed after decapitation of the animals. The tissue was weighed and homogenized in the cold in 10 ml of 10% TCA, and the epinephrine was extracted with aluminum oxide according to the method of Sourkes and Murphy.[14] For the quantitative determinations in the acid extract, the fluorometric method described by Shore and Olin[15] was used. The readings were done in a Farrand or in a Zeiss spectrofluorometer. The activation wavelength was 400 mμ and the emission wavelength was 520 mμ.

RESULTS

Effect of 3-AP on blood glucose levels in several experimental conditions. The effect of i.p. administration of 3-AP on blood glucose concentration and on hepatic and muscular glycogen is shown in Table 1. The previously reported hyperglycemia was accompanied by a significant decrease of muscular and liver glycogen. The hyperglycemia induced by 3-AP in young rats was less notable than that observed in adult rats (Table 1).

It is noteworthy that when 3-AP was administered simultaneously with the ganglionic blocking agent mecamylamine at the doses indicated in Table 2, the 3-AP-induced hyperglycemia was prevented (Table 2).

The intracisternal administration of 3-AP at a dose of 100 μmole/rat (weighing 110–120 g) produced a significant hyperglycemia (Table 3). It is interesting that the i.p. administration of 3-AP at the same dose did not exhibit such effect. Immediately after the intracisternal injection of 3-AP, the animals showed depression, weakness of posterior limbs, and respiratory difficulties. In other experiments rats were injected to study the lethality of 3-AP under these conditions. The animals died within 1–7 hr.

Effect of 3-AP-NAD on blood glucose concentration. The intracisternal administration of 3-AP-NAD at a dose of 1 μmole/rat (weighing 100–110 g) induced hyperglycemia in 15 min (Table 4). It is interesting that neither the i.p. administration of this

TABLE 1. EFFECT OF 3-AP (800 MG/KG) ON BLOOD GLUCOSE CONCENTRATION, HEPATIC GLYCOGEN AND MUSCULAR GLYCOGEN IN ADULT AND YOUNG RATS*

Rats	Blood glucose (mg/100 ml)	Hepatic glycogen (g/100 g)	Muscular glycogen (g/100 g)
Control (adults)	118·8 ± 5·36 (24)	2·57 ± 0·20 (17)	0·19 ± 0·02 (9)
Treated (adults)	261·8 ± 27·6† (22)	1·30 ± 0·17† (23)	0·10 ± 0·01† (9)
Control (8 days old)	100·0 ± 5·55 (9)		
Treated (8 days old)	148·0 ± 6·74†‡ (8)		

* The values are mean ± S.E.M.; number of animals is in parentheses. The animals were sacrified 2 hr after 3-AP administration.
† $P < 0·001$ (difference from control values).
‡ $P < 0·001$ (difference from adult treated rats).

TABLE 2. EFFECT OF SIMULTANEOUS ADMINISTRATION OF 3-AP (800 MG/KG) AND MECAMYLAMINE (10 MG/KG) ON BLOOD GLUCOSE LEVELS IN ADULT RATS*

Control	3-AP	Mecamylamine	3-AP + mecamylamine
106·2 ± 7·69 (7)	189·0 ± 17·6† (8)	101·4 ± 6·30 (7)	101·8 ± 6·13 (10)

* The values are mg/100 ml, mean ± S.E.M.; number of animals is in parentheses. The animals were sacrified 2 hr after the administration of the drugs.
† $P < 0·001$ (difference from control value and difference from 3-AP + mecamylamine-treated rats)

TABLE 3. EFFECT OF INTRACISTERNAL ADMINISTRATION OF 3-AP ON BLOOD GLUCOSE CONCENTRATION IN ADULT RATS*

Route of administration (100 μmole/rat)	Control	Treated
Intracisternal	107·6 ± 5·07 (11)	178·0 ± 9·40† (12)
Intraperitoneal		94·0 ± 6·40‡ (7)

* The values are mg/100 ml, mean ± S.E.M.; number of animals is in parentheses. The rats were sacrified 30 min after the administration of 3-AP.
† $P < 0·001$ (difference from control value).
‡ $P < 0·001$ (difference from intracisternally treated rats).

analogue at the same dose nor the intracisternal administration of 1 μmole 3-AP showed any effect on blood glucose levels (Table 4).

Effect of 3-AP on the epinephrine content of the adrenal glands. The results of the determination of epinephrine in adrenal glands from control and 3-AP-treated rats

are shown in Table 5. Although in all determinations the content of epinephrine was lower in the treated than in the control animals, the difference was not significant. Similar results were obtained with doses of 3-AP of 100 mg/kg, 1–5 hr after administration of the drug.

TABLE 4. EFFECT OF INTRACISTERNAL ADMINISTRATION OF 3-AP-NAD ON BLOOD GLUCOSE CONCENTRATION IN ADULT RATS*

Compound injected	Route of administration (1 μmole/rat)	Control	Treated
3-AP-NAD	Intracisternal	108·0 ± 6·90 (5)	160·0 ± 8·85† (7)
3-AP	Intracisternal	111·5 ± 5·90 (8)	119·6 ± 4·53‡ (13)
3-AP-NAD	Intraperitoneal	118·0 ± 2·29 (7)	111·0 ± 7·00‡ (9)

* The values are mg/100 ml, mean ± S.E.M.; number of animals is in parentheses. The animals were sacrified 15 min after the administration of the drugs.

† $P < 0{\cdot}01$ (difference from control value).

‡ $P < 0{\cdot}001$ (difference from intracisternally 3-AP-NAD-treated rats).

TABLE 5. EPINEPHRINE CONTENT IN ADRENAL GLANDS OF CONTROL RATS AND OF RATS TREATED WITH 3-AP (800 MG/KG)*

Control	Treated	P (*t* test)
64·3 ± 5·05 (8)	54·3 ± 3·20 (8)	> 0·05

* The values are μg/100 mg wet tissue, mean ± S.E.M.; number of animals is in parentheses. The animals were sacrificed 2 hr after 3-AP administration.

DISCUSSION

On the basis of the lack of effect of 3-AP on blood glucose concentration in adrenalectomized animals, it has been suggested previously that the hyperglycemic action of 3-AP is secondary to the liberation of cathecholamines from the adrenal glands.[10] The results of the present paper support this conclusion: 3-AP administration induced a decrease in glycogen concentration in liver and muscle similar to that observed after the administration of epinephrine[16] (Table 1). Furthermore, the ganglionic blocking agent mecamylamine, which prevents the release of cathecholamines from the adrenal medulla,[17] inhibited the hyperglycemic action of 3-AP (Table 2). The lack of effect of 3-AP on the epinephrine content in adrenal glands could be explained, assuming that the drug acts upon the labile pool of cathecholamines, which in brain,[18, 19] ganglia[20, 21] and heart[22–24] is known to be small in comparison to the stable pool. This is probably also the explanation of the fact that sympathetic stimulation does not cause a decrease in the concentration of cathecholamines in some tissues.[25] Since this labile pool is thought to be the physiologically active one,[21, 26] this interpretation agrees with the postulate that the release of epinephrine from the adrenal medulla is not the result of a direct effect on it, but it is secondary to a stimulus originated by its action on the central nervous system (see below).

The data presented here also indicate that the release of epinephrine induced by 3-AP is mediated by the central nervous system. Since the ganglionic blocking agents are known to prevent the release of cathecholamines, the observed inhibition of the hyperglycemic effect of 3-AP by mecamylamine (Table 2) was to be expected if the liberation of epinephrine from the adrenal medulla were due to nervous impulses reaching it from other nervous structures. Furthermore, the intracisternal injection of 3-AP, at a dose which had no effect by i.p. administration, produced hyperglycemia in a short period of time (Table 3).

The finding that very small doses of 3-AP-NAD injected intracisternally also produced hyperglycemia, but the same dose of 3-AP did not, indicates that the probable mechanism of action of 3-AP involves the formation of NAD analogues in the central nervous system. This idea agrees with the findings of Kaplan and Ciotti,[6] which indicate that 3-AP can form pyridine nucleotide analogues in an exchange reaction catalyzed by NADase. In this regard, it would seem important to emphasize that the NADase activity is higher in hypothalamus than in any other cerebral area studied.[11]

It is known that NADase activity is lower in young rats than in adult rats[27] and that the histological lesions induced by 3-AP in brain are also less severe in young rats than in adult animals.[1] Thus, it would be expected that in young rats, with a low level of NADase, the hyperglycemia produced by 3-AP would be less than in adult rats. This prediction was confirmed in the experiments with 8-day-old rats (Table 1), which support the idea that the 3-AP-NAD formed after 3-AP administration is responsible for the effects of 3-AP. The fact that the previous administration of nicotinamide prevents the hyperglycemia induced by 3-AP[10] constitutes further support for this conclusion.

It is possible that the reported inhibition of certain dehydrogenases in the central nervous system by pyridine nucleotide analogues[8, 9] initiates a series of biochemical events which would result in the postulated nervous stimulation of the adrenal glands and in the histological lesions observed in hypothalamus and other areas in brain after the administration of 3-AP. However, with the available data, this statement can only be a speculation. Much work has to be done before the relation among biochemical, physiological and morphological lesions can be understood.

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