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Participation of adrenal medulla in 6-amino-nicotinamide-induced hyperglycaemia in the rat

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In fasted rats 6-aminonicotinamide (6-AN) produced delayed hyperglycaemia, the peak effect being seen by 7 h. Fasting plasma insulin concentrations were not significantly altered but liver glycogen concentrations were decreased following treatment with 6-AN. Adrenalectomy, demedullation and pretreatment with reserpine, phentolamine, nicotinamide and nicotinic acid completely blocked the hyperglycaemic response whereas guanethidine and propranolol or oxprenolol were ineffective. Catecholamine concentrations in the adrenal venous plasma were markedly increased by treatment with 6-AN, the peak effect being seen by 5 h. It is concluded that adrenal medullary release, which is slow in onset, is mainly responsible for the development of sustained hyperglycaemia in the rat.

6-Aminonicotinamide (6-AN), an antimetabolite of pyridine nucleotide synthesis (Dietrich et al 1958), has been reported to induce hyperglycaemia in the rat (Schultz et al 1966; Ammon & Steinke 1972) the exact mechanism for which is not clear. It has been attributed to inhibition of insulin release (Ammon & Steinke 1972; Ammon et al 1973) and to adrenal medullary discharge (Schultz et al 1966). To obtain further insight into how 6-AN induces hyperglycaemia we have used drugs affecting sympathetic function, phentolamine, propranolol, oxprenolol and guanethidine and a drug that influences the adrenal meduallary catecholamine stores (reserpine). We have also used nicotinamide and nicotinic acid as they have been reported to block some of the effects of 6-AN (Johnson & McColl 1955, 1956).

METHODS

Female rats, Charles Foster strain, 180–200 g, were fasted for 16–18 h. Blood samples were collected through the orbital sinus at different times in unanaesthetized animals. Blood sugar was estimated on the Auto-Analyzer using the Hoffman micro-method. Demedullation was performed by making a small incision on the adrenal gland and squeezing out the medullary contents. Adrenalectomized and demedullated rats were used 4 or 5 days after surgery. Adrenalectomized rats were kept on 0·9% NaCl (saline) during this period. Plasma insulin (IRI) was estimated by radioimmunoassay (Hales & Randle 1963) using the kit from the Radiochemical Centre, Amersham, U.K. Human insulin supplied with the kit was used as a standard. Liver glycogen was estimated as described by Roe & Dailey (1948). Catecholamine release, as noradrenaline, was measured during the control period and at different times in the venous effluent from the cannulated main adrenal vein of rats anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.). In these experiments, both jugular veins were cannulated, one for the injection of drug and the other for continuous infusion of heparinized saline (0·1 ml min^{-1}). Adrenal venous blood was collected (approximately 0·5–0·7 ml) in chilled centrifuge tubes. The plasma was separated at 2000 rev min^{-1} and assayed for noradrenaline on the blood pressure of the pithed rat (Kaul & Grewal 1968). All the data were statistically analysed using Student's *t*-test. 6-AN was dissolved in Krebs buffer at pH 3·5. Guanethidine sulphate, phentolamine hydrochloride, nicotinic acid hydrochloride, nicotinamide, oxprenolol and propranolol were dissolved in water. Reserpine was given as Serpasil.

RESULTS

Effect on blood sugar, plasma (IRI) and liver glycogen. 6-AN, at 35 mg kg^{-1} i.p., produced marked hyperglycaemia in the fasted rats. There was a delay of about 3 h in the onset of hyperglycaemia and the peak effect was seen at about 7 h (Fig. 1). We therefore selected 7 h for subsequent studies. Adrenalectomy or demedullation induced small but significant decreases in the blood sugar values compared with intact controls ($P < 0{\cdot}01$–$0{\cdot}001$). Adrenalectomy or demedullation completely blocked the hyperglycaemic response to 6-AN. Pretreatment of adrenalectomized rats with hydrocortisone acetate for four

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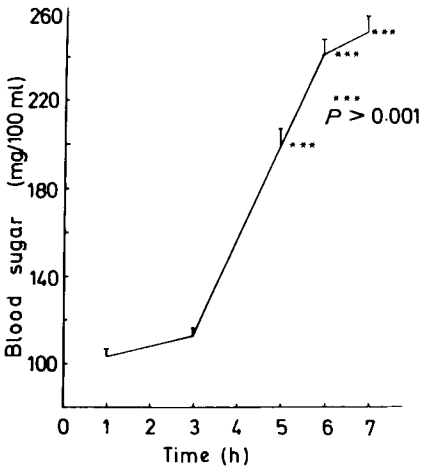

FIG. 1. Blood sugar concentrations in the rat after 6-AN (35 mg kg^{-1} i.p.) at various times. There is a delay in hyperglycaemia and the peak effect is seen by 7 h (n = 12).

days only slightly restored the response to 6-AN (Table 1). In the fasted rats, 6-AN had no significant effect on plasma IRI values but produced a significant reduction in liver glycogen concentrations of non-fasted rats 6 h later (control 31·8 ± 2·1 s.e.m. n = 10; 6-AN 35 mg kg^{-1} 21·1 ± 2·6 mg g^{-1} n = 10, P <0·001).

Effect of reserpine and guanethidine. Pretreatment of rats with reserpine (2 × 2·5 or 4 × 2·5 mg kg^{-1} i.p.) partially or completely blocked the hyperglycaemic response to 6-AN. The blood sugar values in mg 100 ml^{-1} with 6-AN and reserpine + 6-AN were 293·0 ± 7·0 (n=8) and 177·0 ± 11·8 (n=6) respectively. P <0·001. In contrast, guanethidine (2 × 30 mg kg^{-1} s.c.) was ineffective in blocking 6-AN-induced hyperglycaemia. The blood sugar values in mg 100 ml^{-1} with 6-AN alone and guanethidine + 6-AN were 254·0 ± 4·5 (n=8) and 267·0 ± 14·0 (n=10) respectively.

Table 1. Effect of adrenalectomy and demedullation on the hyperglycaemia produced by 6-AN in the rat. All values are the mean ± s.e.m. Figures in parentheses indicate the number of observations. Adrenalectomized and demedullated rats were used 4–6 days after surgery. Hydrocortisone was given 4 × 5 mg kg^{-1} s.c. every day and animals used 24 h after the last injection.

Series		Treatment and dose (mg kg^{-1})	Blood sugar (mg/100 ml)*
I	a.	Control	102 ± 2·8 (10)
	b.	Adrenalectomized control	82 ± 2·3 (4)
	c.	6-AN 35 i.p.	293 ± 3·9 (16)
	d.	Adrenalectomy + 6-AN 35 i.p.	85 ± 3·2 (16)
	e.	Adrenalectomy + Hydrocortisone 4 × 5 s.c. + 6-AN	114 ± 3·86 (10)
II	a.	Control	109 ± 2·35 (16)
	b.	Demedullated control	87 ± 2·25 (16)
	c.	6-AN 35 i.p.	293 ± 5·9 (19)
	d.	Demedullated + 6-AN 35 i.p.	103 ± 3·00 (20)

* Measured 7 h after 6-AN administration
***P < 0·001 I a–b, a–c, c–d; II a–b, a–c, c–d.

Effect of phentolamine, oxprenolol and propranolol. Phentolamine (2 × 20 mg kg^{-1} i.p.) completely inhibited while propranolol and oxprenolol did not modify 6-AN-induced hyperglycaemia. (Table 2).

Table 2. Effect of phentolamine, propranolol and oxprenolol on the hyperglycaemia induced by 6-AN in the rat. All values are the mean ± s.e.m. Figures in parentheses indicate the number of observations. Propranolol, oxprenolol and phentolamine were administered 15 min before 6-AN and again 3 h later. Blood sugar measured 4 h after 6-AN.

Treatment and dose mg kg^{-1}	Blood sugar mg/100 ml
a. Controls	96 ± 3·2 (10)
b. 6-AN 35 i.p.	258 ± 11·0 (10)
c. Propranolol 2 × 20 s.c. + 6-AN 35 i.p.	248 ± 16·0 (9)
d. Phentolamine 2 × 20 i.p. + 6-AN 35 i.p.	85 ± 5·3 (9)
e. Controls	94 ± 3·1 (10)
f. 6-AN 35 i.p.	270 ± 8·8 (10)
g. Oxprenolol 2 × 20 i.p. + 6-AN 35 i.p.	243 ± 8·7 (10)

*** P < 0·001 a–b, b–d, e–f.

Effect of nicotinamide and nicotinic acid. Pretreatment of rats with nicotinamide (250 mg kg^{-1} i.p.) or nicotinic acid (500 mg kg^{-1} i.p.) completely or partially blocked the hyperglycaemic response to 6-AN (P <0·001) whereas nicotinamide or nicotinic acid themselves had no significant effect on blood sugar (Table 3) and neither drug altered the hyperglycaemic response after adrenaline injection. (Control, 79 ± 3·4 s.e.m. n=8; adrenaline 0·1 mg kg^{-1}, 178 ± 7·9 n=8; adrenaline 0·1 + nicotinamide 250 mg kg^{-1}, 161 ± 9·7 n=8; adrenaline 0·1 + nicotinic acid 500 mg kg^{-1}, 184 ± 9·2 mg 100 ml^{-1} n=8).

Effect of 6-AN on the adrenal catecholamine release. There was a significant increase in the catecholamine output (expressed as ng total or ng min^{-1}) starting

Table 3. Effect of nicotinamide and nicotinic acid on the hyperglycaemia induced by 6-AN in the rat. All values are the mean ± s.e.m. Figures in parentheses indicate the number of observations. Nicotinamide and nicotinic acid were administered 0·5 h before 6-AN.

Expt. No.	Treatment and dose mg kg^{-1}	Blood sugar mg/100 ml
1.	a. Controls	81 ± 4·1 (10)
	b. 6-AN 35 i.p.	201 ± 7·6 (10)
	c. Nicotinamide 250 i.p. + 6-AN 35 i.p.	89 ± 3·2 (10)
2.	a. Controls	95 ± 4·3 (10)
	b. 6-AN 35 i.p.	234 ± 7·9 (10)
	c. Nicotinic acid 500 i.p. + 6-AN 35 i.p.	131 ± 8·3 (8)
3.	Controls	88 ± 3·6 (10)
	Nicotinamide 250 i.p.	94 ± 4·8 (10)
	Nicotinic acid 500 i.p.	84 ± 5·6 (12)

$P < 0{\cdot}001$ 1 a–b, b–c, 2 a–b, b–c.

from 1 h after 6-AN administration and by the fifth hour it was about six times higher than the basal values ($P < 0{\cdot}001$) (Fig. 2).

DISCUSSION

The results show that 6-AN-induced adrenaline release from the adrenals is mainly responsible for the development of hyperglycaemia in the rat. This conclusion is based on the following observations: (a) adrenalectomy and adrenal demedullation completely blocked the hyperglycaemic response to 6-AN, (b) 6-AN induced adrenaline release from the adrenals, (c) The α-adrenoceptor blocking drug (phentolamine) blocked the hyperglycaemic effect whereas the β-adrenoceptor blocking drugs oxprenolol or propranolol were ineffective, (d) reserpine, a known adrenal catecholamine depletor (Kirpekar et al 1958), inhibited the hyperglycaemic response to 6-AN whereas guanethidine, which does not deplete the adrenal catecholamine stores (Cass et al 1960), was ineffective, (e) a decrease in liver glycogen was seen after 6-AN-following adrenaline release. Although phentolamine has been reported to stimulate insulin secretion in the rat, this effect is of short duration (Furman & Tayo 1974; Talwalker et al 1979). Therefore, it is reasonable to assume that the antagonism to the hyperglycaemic effect of 6-AN is due to antagonism of catecholamines released by 6-AN and not due to the antagonistic effect of elevated insulin concentrations since increased insulin concentrations are not seen up to 7 h after phentolamine treatment.

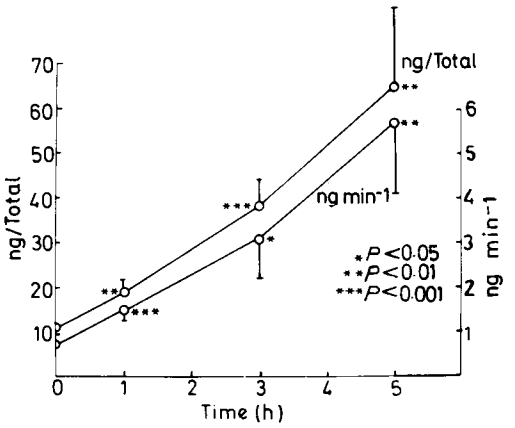

FIG. 2. Catecholamine release from the rat adrenal gland following 6-AN (35 mg kg^{-1} i.p.). There is a marked and sustained increase in the output which reaches its maximum by about 5 h.

The inability of β-adrenoceptor blocking drugs to antagonize 6-AN-induced hyperglycaemia was not surprising since those drugs even in large doses have been reported to have little effect in adrenaline-induced hyperglycaemia in the rat (Claussen & Noach 1960; Hornbrook & Brody 1963; Lei & McCutcheon 1964). It appears that adrenal cortical hormones do not play any significant role in 6-AN-induced hyperglycaemia since hydrocortisone did not restore the hyperglycaemic effect of 6-AN in the adrenalectomized rats. This is further supported by the observations of Schultz et al (1966) that there was no significant change in the adrenal corticosterone values following 6-AN treatment. We found 6-AN had no significant effect on the fasting plasma IRI concentrations thus confirming similar observations of Ammon & Steinke (1972). However, 6-AN inhibits glucose-induced insulin release (in vivo and in vitro) in the rat (Ammon & Steinke 1972) which would suggest that the sustained hyperglycaemia observed with 6-AN may be due to the combined effect of stimulation of adrenaline release from the adrenals and inhibition of insulin release. Administration of 6-AN results in the formation of the 6-amino analogue of NADP which, by inhibiting 6-phosphogluconate-dehydrogenase, blocks the pentose phosphate shunt (PPS) (Herken et al 1969). The PPS may be involved in catecholamine synthesis in the adrenal medulla (Jansson et al 1977) or glucose-induced insulin release in the pancreas (Ammon & Steinke 1972). Therefore, the sustained hyperglycaemic effect observed with 6-AN may be due to the inhibition of the PPS in adrenal medulla as well as pancreas. This is supported by our observation that

concurrent administration of nicotinic acid or nicotinamide had no effect on blood sugar concentration but could block the 6-AN-induced hyperglycaemia. Further, the hyperglycaemic effect of adrenaline could not be antagonized by nicotinic acid or nicotinamide.

The delay observed in the production of hyperglycaemia in the rat suggests that 6-AN activity was contingent upon metabolic transformation in vivo probably by being incorporated in pyridine nucleotides in place of nicotinamide. It has also been observed that the accumulation of 6-phosphogluconate (which reflects the inhibition of PPS) in the adrenal medulla following 6-AN administration is also delayed by 3–4 h in the rat (Jansson et al 1977). These observations suggest that the delayed hyperglycaemic response seen with 6-AN may be due to the delayed release of significant amounts of catecholamines from the adrenal gland. Our results with catecholamine release provide a direct support to the observations of Schultz et al (1966) who showed marked depeletion of adrenal catecholamines by 3 h following 6-AN administration.

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REFERENCES

Ammon, H. P. T., Steinke, J. (1972) Diabetes 21: 143–148

Ammon, H. P. T., Patil, T. N., Steinke, J. (1973) Biochem. Biophys. Acta 297: 352–367

Cass, R., Kuntzman, R., Brodie, B. B. (1960) Proc. Soc. Exp. Biol. Med. 103: 871–882

Claussen, N., Noach, E. L. (1960) Arch. Int. Pharmacodyn. 126: 332–340

Dietrich, L. S., Friedland, J. M., Kaplan, A. (1958) J. Biol. Chem. 233: 964–968

Furman, B. L., Tayo, F. M. (1974) J. Pharm. Pharmacol. 26: 512–517

Hales, C. N., Randle, P. J. (1963) Biochem. J. 88: 137–146

Herken, H., Lange, K., Kolbe, H. (1969) Biochem. Biophys. Res. Commun. 36: 93–100

Hornbrook, K. R., Brody, T. M. (1963) Biochem. Pharmacol. 12: 1407–1415

Jansson, S. E., Gripenberg, J., Harkonen, M. (1977) Acta Physiol. Scand. 99: 467–475

Johnson, W. J., McColl, J. D. (1955) Science 122: 834

Johnson, W. J., McColl, J. D. (1956) Fed. Proc. 15: 284

Kaul, C. L., Grewal, R. S. (1968) J. Pharm. Sci. 57: 1741–1744

Kirpekar, S. M., Goodlad, G. A., Lewis, J. J. (1958) Biochem. Pharmacol. 1: 232–233

Lei, B. W., McCutcheon, R. S. (1964) J. Pharm. Sci. 53: 503–506

Roe, J. H., Dailey, R. E. (1948) Anal. Biochem. 15: 245–250.

Schultz, G., Senft, G., Losert, W., Schacht, U. (1966) Arch. Exp. Path. U. Pharmacol. 253: 345–354

Talwalker, P. K., Kaul, C. L., Grewal, R. S. (1979) J. Pharm. Pharmacol. 31: 598–600