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THE EFFECT OF NICOTINE ON BLOOD GLUCOSE LEVELS AND PLASMA NON-ESTERIFIED FATTY ACID LEVELS IN THE INTACT AND ADRENALECTOMIZED CAT

BY

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The work described in this paper is an investigation of the effects of nicotine, administered in a series of small intravenous injections, on blood glucose and non-esterified fatty acid levels in the cat. Armitage (1965), when describing the cardiovascular effects of smoking, said that the intake of nicotine during smoking was probably similar to that resulting from a series of small intravenous injections. There are several reports in the literature describing the effects of nicotine on blood sugar levels (see Larson, Haag & Silvette, 1961a). Most authors have found that, after the administration of nicotine, blood glucose levels rise. But they do not all agree on the part the adrenal gland plays in this effect. Inaba & Oikawa (1930) and Leloir (1933, 1934) observed that in the dog and rabbit the elevation of blood glucose did not occur when the adrenal gland had been removed. Others (Hazard & Vaille, 1935; Wada, 1935) noted that following adrenalectomy in the dog the rise was less pronounced, and Wada (1935) claimed that in the rabbit the adrenal gland played no part in the hyperglycaemia caused by nicotine. The discrepancies in these results may be due to several factors, such as the species, the route of administration, and the dose of nicotine.

The effect of nicotine on non-esterified fatty acid levels was first reported by Kershbaum, Bellet, Dickstein & Feinberg (1961). They observed that, after the infusion of a solution containing nicotine (20 μg/kg/min) into the anaesthetized dog, there occurred a rise in serum non-esterified fatty acid levels.

In the experiments to be described, nicotine was administered in doses from 1 to 100 μg/kg. The effects of such doses have been measured both in intact and in adrenalectomized animals.

METHODS

Experimental procedures

Cats weighing between 2.0 and 3.5 kg were deprived of food for 12 hr before use. They were anaesthetized with intravenous chloralose (80 mg/kg) after induction with ether. Blood pressure was recorded from either the left carotid or the left femoral artery and blood samples were obtained from either of the femoral arteries or from the left carotid artery. The arteries were cannulated with polyethylene tubing. Nicotine was administered through a polyethylene cannula in the right femoral vein. In the experiments using the denervated nictitating membrane preparation, the right superior cervical ganglion was removed aseptically

10 to 17 days before the experiment. Nictitating membrane contractions and blood pressure were recorded on a smoked drum. To study the effects in the absence of the adrenal glands, all the blood vessels to and from the adrenal glands were tied off as quickly as possible and the glands were removed intact. The body temperature was maintained at 37.5° C by placing the animal on an electrically heated operating table which was controlled by a mercury contact thermometer situated in the rectum of the cat. Control blood samples (three or four) were taken at 15 min intervals, the final control sample being taken 1 min before the first injection of nicotine. The preparations were left for at least 1 hr after setting up before samples of blood were taken for estimations in order to allow the effects of the initial stress to subside.

Nicotine administration

Ten injections of nicotine were administered at 1 min intervals and a blood sample was taken 10 min after the first injection (that is, 1 min after the tenth injection). Subsequent samples were taken at 20, 30, 45 and 60 min after the first injection. The nicotine solutions, made up in 0.9% saline, were administered in a volume of 0.1 ml. and washed in with a volume of 0.4 ml. of 0.9% saline. Nicotine hydrogen tartrate was used, and all the doses are expressed in terms of the amount of free base administered.

Glucose estimations

The glucose content of 0.05 ml. samples of blood taken from the arterial cannula was measured by the colorimetric method of Asatoor & King (1954) using an EEL Colorimeter with a 607 filter (orange).

Non-esterified fatty acid estimations

A 2 ml. sample of arterial blood was taken and centrifuged for 5 min. The non-esterified fatty acid content of a 1 ml. aliquot of the plasma was measured by the method of Dole (1956).

RESULTS

In thirty-two experiments the level of blood glucose immediately before the administration of either saline control or nicotine injections was 148 ± 8.66 mg per 100 ml. (mean and standard error). For plasma non-esterified fatty acid in twenty-seven experiments it was 517 ± 33.6 μequiv/l.

A summary of the results is shown in Table 1. This table shows the proportion of experiments in which a rise of at least 10% occurred in either the glucose or non-esterified fatty acid levels in the first hour after the administration of nicotine. The results may be considered in more detail as follows (see also Table 2): all headings refer to ten injections of the dose stated given at 1 min intervals.

TABLE 1

PROPORTION OF CATS RESPONDING TO TEN INTRAVENOUS INJECTIONS OF NICOTINE GIVEN AT 1 MIN INTERVALS BY A RISE IN NON-ESTERIFIED FATTY ACID AND GLUCOSE LEVELS OF AT LEAST 10%

Each pair of numbers gives the number of cats which responded and the number tested

Dose of nicotine per injection (μg/kg)	Response in cats			
	Intact		Adrenalectomized	
	Non-esterified fatty acids	Glucose	Non-esterified fatty acids	Glucose
1	1/3	1/4	—	—
2	1/2	1/3	—	—
5	3/3	3/5	—	—
10	6/7	6/8	0/2	0/2
20	4/6	3/4	3/4	0/1
50	3/4	6/6	2/2	1/3
100	0/2	2/2	1/1	2/2

Intact animals

0.9% *saline* (0.5 *ml./injection*). In control experiments saline was without effect on blood glucose levels, plasma non-esterified fatty acid levels and the denervated nictitating membrane.

Nicotine, 1 *μg/kg*. In only one experiment did this dose have any effect and this preparation may be considered abnormally sensitive.

Nicotine, 2 *μg/kg*. As with 1 μg/kg this dose was not effective in most experiments. In one experiment the denervated nictitating membrane contracted, the blood pressure rose and an increase in blood glucose was measured.

Nicotine, 5 *μg/kg*. In all experiments non-esterified fatty acid levels rose whereas in only three out of five was the glucose elevated. A record showing the contractions of the nictitating membrane and the rise in blood pressure after the administration of this dose of nicotine is shown in Fig. 1. Table 3 gives the experimental results obtained with this dose of nicotine.

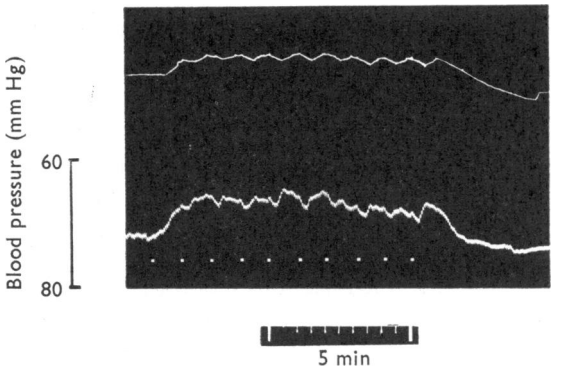

Fig. 1. Effect of nicotine on nictitating membrane contractions and blood pressure. Cat, 3.2 kg, chloralose anaesthesia. The right superior cervical ganglion was removed aseptically 12 days earlier. Top trace: nictitating membrane contractions; bottom trace: blood pressure (left femoral artery). The white dots indicate intravenous injections into the right femoral vein of nicotine, 5 μg/kg.

Nicotine, 10 *μg/kg*. This dose was effective in elevating both non-esterified fatty acid and blood glucose. There were marked effects on the blood pressure and contractions of the nictitating membrane occurred.

Nicotine, 20 *and* 50 *μg/kg*. Both these doses had similar effects. The glucose rose rapidly and, though the non-esterified fatty acid levels normally rose, the effect was often transient with the levels falling as the glucose levels rose (Fig. 2).

Nicotine, 100 *μg/kg*. In some experiments this dose was toxic and the animals did not survive the ten injections. Glucose levels rose rapidly, and the non-esterified fatty acid levels either remained the same or fell.

Adrenalectomized animals

Following adrenalectomy control non-esterified fatty acid levels invariably fell. On the other hand changes in glucose levels were variable, and probably depended upon the amount of manual stimulation of the adrenal glands which occurred when they were removed.

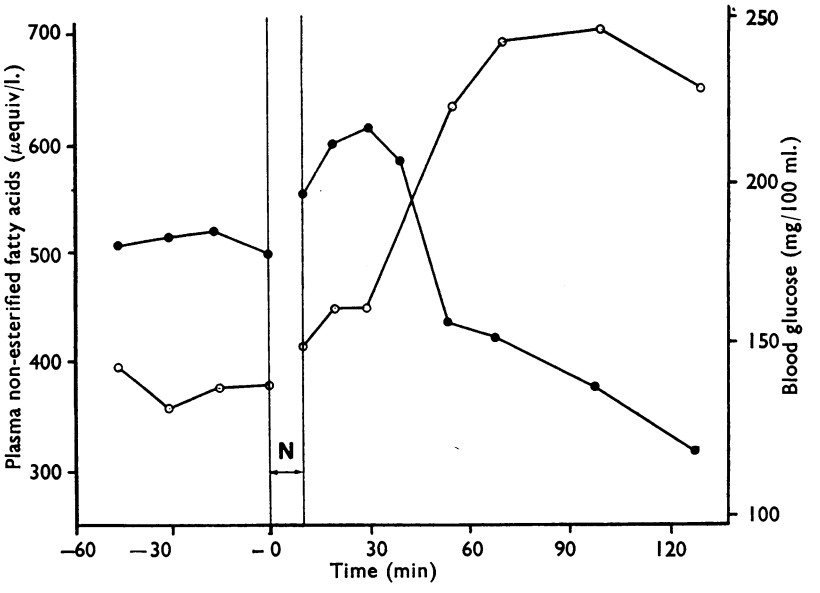

Fig. 2. Changes in blood glucose and plasma non-esterified fatty acid levels after the administration of nicotine. Cat, 2.8 kg, chloralose anaesthesia. ● = plasma non-esterified fatty acid level; O = blood glucose level; N = ten injections of nicotine (20 μg/kg) at 1 min intervals.

Nicotine, 10 *μg/kg*. This dose had no effect on non-esterified fatty acid or glucose levels or on the denervated nictitating membrane, in contrast to the effects in the intact animal.

Nicotine, 20 *μg/kg*. At this dose the non-esterified fatty acid levels rose and effects were observed on the nictitating membrane and blood pressure.

Nicotine, 50 *μg/kg*. The non-esterified fatty acid levels rose greatly with this dose, but only in one experiment was there an effect on the blood glucose and this was slight.

Nicotine, 100 *μg/kg*. This dose elevated the glucose levels to a greater extent than the non-esterified fatty acid levels.

DISCUSSION

The resting levels of blood glucose and non-esterified fatty acids are of interest as this appears to be the first occasion on which they have been measured simultaneously in the cat. It should be noted that these levels were measured in anaesthetized animals which had undergone considerable stress (for example, induction of anaesthesia with ether) and as a result the glucose levels may have been elevated and the non-esterified fatty acid levels depressed compared with those in conscious animals.

The results indicate that intravenous injections of nicotine administered to the cat in comparatively low doses are capable of affecting the mobilization of both glucose and non-esterified fatty acids. The results also illustrate the relationship between glucose and non-esterified fatty acid levels.

In those experiments in which contractions of the denervated nictitating membrane were recorded at the same time as glucose and non-esterified fatty acid levels were measured,

TABLE 2

PERCENTAGE RISE IN BLOOD GLUCOSE AND PLASMA NON-ESTERIFIED FATTY ACID LEVELS IN FIRST 10 MIN AND AT END OF FIRST HOUR AFTER ADMINISTRATION OF NICOTINE TO CATS

Ten injections of each dose were given. + = 10–19% rise; ++ = 20–39% rise; +++ = 40–59% rise; ++++ >60% rise; − = 10–19% fall; −− = 20–39% fall; −−− = 40–59% fall; −−−− >60% fall; 0 <10% change

Dose of nicotine per injection (μg/kg)	Expt.	Rise (%) in blood or plasma level of: Glucose 1st 10 min	Glucose 1 hr	Non-esterified fatty acids 1st 10 min	Non-esterified fatty acids 1 hr
1	1	0	0	0	0
	2	0	0	0	0
	3	0	++++	+	−−
	4	0	0		
2	5	0	0	0	0
	6	0	0	+	0
	7	+	++		
5	8	0	+++	+	−
	9	−	−	+	++
	10	−−	−	0	++
	11	0	++		
	12	0	++		
10	13	0	0	++	0
	14	0	+	0	0
	15	+	0	+	0
	16	+	++	++	−
	17	−	−	0	++
	18	0	+	++	0
	19	0	+		
	20	++	+		
	21			+	−−
20	22	++	++++	++	−
	23	++	++	−	−−−
	24	+	+++	0	−−−−
	25	0	0		
	26			++++	+++
	27			+++	+
	28			+	−
50	29	++	+++	−	−−
	30	+	0	++	−−−
	31	++	0	++++	0
	32	++	−	+++	0
	33	+++	0		
	34	+	0		
100	35	0	++++	−	0
	36	+++	−		
	37			0	0

TABLE 3

EFFECT OF TEN INJECTIONS OF NICOTINE (5 μg/kg) GIVEN INTRAVENOUSLY AT 1 MIN INTERVALS ON NON-ESTERIFIED FATTY ACID AND GLUCOSE LEVELS

Expt.	Blood glucose concentrations (μg/100 ml.) Control	10 min after injection	1 hr after injection	Plasma non-esterified fatty acid concentrations (μequiv/l.) Control	10 min after injection	1 hr after injection
1	134	136	180	751	850	634
2	85	72	75	548	641	759
3	73	54	60	590	642	720
4	170	186	206	—	—	—
5	96	99	115	—	—	—

elevation of these levels by nicotine occurred only when the membrane contracted. It was assumed that the contraction of the membrane was due to release of adrenaline from the adrenal medulla. In the absence of the adrenal glands low doses of nicotine were without effect on glucose and non-esterified fatty acid levels and there was no contraction of the membrane. This suggests that at low doses the mobilizing action of nicotine is entirely due to stimulation of the adrenal gland. The results suggest that the threshold dose of nicotine necessary to mobilize non-esterified fatty acid is slightly lower than that needed to mobilize glucose. With ten doses 20 μg/kg and above in the intact animal the mobilization of non-esterified fatty acids was often of only short duration and Fig. 2 shows the fall in non-esterified fatty acids which occurred in the presence of marked hyperglycaemia. This relationship between non-esterified fatty acid levels and glucose levels is also illustrated in Table 2 (experiments 3, 8 and 16) where, after the initial increase in non-esterified fatty acid levels, the levels were depressed by the marked hyperglycaemia which slowly developed.

In the adrenalectomized preparations there was no effect on either non-esterified fatty acid or glucose levels with ten doses of nicotine below 20 μg/kg. At this and higher doses the denervated nictitating membrane contracted, presumably due to the nicotine stimulating ganglia throughout the body and releasing noradrenaline from the sympathetic post-ganglionic nerve endings. Noradrenaline can mobilize non-esterified fatty acids and is as active as adrenaline (Havel & Goldfien, 1959) whereas it has a low hyperglycaemic action compared with adrenaline (Trendelenburg, 1953; Ellis, 1956). Stimulation of the sympathetic nerve supply to the liver can mobilize glycogen to glucose and central stimulation can also affect blood glucose levels (Keele & Neil, 1961). It was observed that with the higher doses of nicotine there was marked central nervous stimulation as recorded by hyperventilation and the characteristic ear twitch which occurs when nicotine is injected directly into the lateral ventricle (Armitage, Milton & Morrison, 1965). In the adrenalectomized preparation, ten nicotine doses of 50 μg/kg were very effective in raising plasma non-esterified fatty acid levels, but had little effect on blood glucose levels. Any noradrenaline released by sympathetic stimulation would be expected to have little hyperglycaemic effect, and non-esterified fatty acid mobilization would therefore occur without depression due to mobilization of glucose. On the other hand, ten nicotine doses of 100 μg/kg in the adrenalectomized preparation cause sufficient sympathetic stimulation to mobilize glucose and at the same time the non-esterified fatty acid stimulation is less marked. This again illustrates the interrelationship between the two substances. The effects were not sustained; this may have been due either to ganglionic blockade by the nicotine or to rapid deterioration of the preparations after adrenalectomy.

In previous work describing the hyperglycaemic actions of nicotine relatively large doses of nicotine were administered. Thus Burstein & Goldenberg (1928) found that 0.2 to 1 mg/kg of nicotine subcutaneously causes a 20% increase in blood glucose levels in the dog, and Inaba & Oikawa (1930) found that the minimum effective hyperglycaemic subcutaneous dose in the rabbit is 0.5 mg/kg. Boldyreff (1935) found no effect on blood glucose levels in the dog with 0.4 mg/kg and Kobayashi (1937) administered 5 to 100 mg/kg subcutaneously to the rabbit to raise the blood glucose by 40 to 100%. Watanabe (1935) found that intravenously 0.5 to 1 mg/kg increases the blood glucose in the dog.

In considering the effect of drugs on the body the route of administration is of considerable importance. This is particularly true of nicotine; for example the LD50 for nicotine

administered subcutaneously is fifteen times greater than the LD50 for nicotine administered intravenously (Larson *et al.*, 1961b). Routes of administration such as the subcutaneous may produce blood levels of nicotine similar to those resulting from a slow intravenous infusion of nicotine. Armitage & Milton (1965), however, showed that large amounts of nicotine can be infused slowly into the vein of the spinal cat without contracting the nictitating membrane, although a much smaller intravenous injection causes a large contraction. The contraction of the nictitating membrane was considered to result from release of adrenaline from the adrenal gland. An effect on blood glucose could best be accomplished by a sustained secretion of adrenaline and this may be most easily brought about either by a large dose of nicotine administered subcutaneously or by several small injections intravenously, as in the experiments described here.

Hazard & Vaille (1935) considered from their results that in the dog the action of nicotine on blood glucose is twofold, both an adrenal and an extra-adrenal factor being normally operative. The results described here in the cat agree with this. However, it would seem that at threshold doses the effect is purely adrenal and that only at high doses do extra-adrenal factors play a role.

Finally, are the results reported here of any relevance with regard to smoking? Armitage (1965) observed that the cardiovascular effects of smoking are similar to a series of intravenous injections of nicotine given rapidly at approximately 1 min intervals. He also suggested that in a human smoker the amount absorbed at each puff was equivalent to an intravenous injection of 1 to 2 μg of nicotine per kg body weight. The threshold dose of nicotine to elevate non-esterified fatty acid and glucose levels in these experiments was of the order of 1 to 5 μg/kg which is considered to be within the smoking range. The results of the higher doses of nicotine are probably not of importance with reference to smoking, though they provide interesting information on the interrelationship between non-esterified fatty acids and glucose and the mode of action of nicotine in affecting these substances.

SUMMARY

1. Blood glucose and plasma non-esterified fatty acid levels have been estimated in the anaesthetized cat. Blood glucose levels were 148 ± 8.66 mg/100 ml., and plasma non-esterified fatty acid levels were 517 ± 33.6 μequiv/l. (means and standard errors).

2. The effects of several rapid intravenous injections of nicotine on the two blood levels have been measured.

3. At low doses (ten injections of 1 to 10 μg/kg) nicotine elevates both glucose and non-esterified fatty acid. This effect is abolished by adrenalectomy.

4. At high doses of nicotine (ten injections of 20 to 100 μg/kg) marked elevation of blood glucose occurs, but the effect on non-esterified fatty acid levels is transient. Following adrenalectomy, the effects on glucose are much reduced, whereas elevation of non-esterified fatty acid occurs. This illustrates the depression of non-esterified fatty acid mobilization in the presence of hyperglycaemia.

5. The low doses of nicotine are considered to be in the smoking range and therefore any glucose or non-esterified fatty acid mobilizing effects of smoking would result entirely from stimulation of the adrenal gland.

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