The Effect of a New Diuretic, Triflocin (4-(α,α,α-trifluoro-*m*-toluidino)-Nicotinic Acid) on Blood Glucose in the Rat and Dog (34082)

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The hyperglycemic action of most of the commonly used diuretics is a well established, undesirable effect (1). Although it was initially believed to be inherent in the thiazide structure (2), several investigators have revealed hyperglycemic activity in a number of structurally diverse diuretics (1, 3, 4, 5). In addition, a relationship between kaliuresis and hyperglycemia has been precluded by the demonstration that triamterene, an antikaliuretic agent, and two nondiuretic chemical analogues of chlorothiazide, diazoxide and EX 4877,[1] can increase blood glucose concentrations (1). Since it is impossible to predict whether or not a diuretic will induce hyperglycemia, the present study was undertaken with triflocin,[2] a new, structurally novel diuretic, which can produce a diuresis similar in magnitude to that evoked by furosemide and ethacrynic acid (6–8).

Methods. Effects of a single dose of triflocin on resting blood glucose. A dose-response study was conducted in male Wistar rats weighing 230–260 g. All were fed a cube diet and water *ad libitum* up to the time of the experiment. A blood sample was obtained by puncture of the retrobulbar venous plexus (9) under light ether anesthesia and immediately afterward triflocin was administered orally as a suspension in 2 ml/kg of 2% starch. The control group of animals received only starch. Additional blood samples were then obtained at 1, 2, 4, and 6 hr after administration of drug. The doses of triflocin used were 10, 25, 50, 100, 200, and 400 mg/kg. Blood glucose was determined utilizing the standard "Autoanalyzer" method (10).

A dose-response study was also carried out in female beagle dogs. The animals were not fed for approximately 18 hr prior to the experiment. After collecting an initial blood sample from the cephalic vein, the drug was administered orally in a gelatin capsule and blood samples were obtained after 1, 2, and 6 hr. Doses of 10, 50, 100, and 200 mg/kg were used. Each dose was administered to four dogs.

Oral glucose tolerance test in rats. Oral glucose tolerance tests were performed on starved male Wistar rats after single oral or subcutaneous doses of triflocin and after daily oral doses for 15 consecutive days. The tests were performed by giving each rat an aqueous solution containing 1.75 g of glucose/kg of body weight by stomach tube. Blood was obtained prior to glucose loading and at various intervals after loading by puncture of the retrobulbar venous plexus. In some experiments the drug was given 2 hr before glucose loading so that the glucose tolerance testing would coincide with the time of peak diuresis and in other experiments the drug was given immediately before the glucose load. The rats in these groups were starved for 18 hr prior to the experiment.

Intravenous glucose tolerance tests in rats. A single dose of triflocin was administered to male Wistar rats 2 hr prior to the intravenous injection of 250 mg of glucose (as 5% dextrose in sterile water) per kg body weight.

[1] 3, 4-dihydro-2-methyl-3-(β-oxopropyl)-7-sulfamyl-6-trifluoromethyl-2H-1,2,4-benzothiadiazine-1,-1-dioxide-1-phthalazinylhydrazone.

[2] Triflocin was previously referred to as CL 65, 562.

The glucose was administered via the femoral vein after a cut-down under light ether anesthesia. After injection the incision was closed with wound clips. Blood samples were obtained by puncture of the retrobulbar plexus before and at various intervals after glucose loading.

Data were analyzed statistically by methods found in Snedecor (11).

Results. Single doses of triflocin in rat and dog. When nonfasted rats were given single oral doses of triflocin ranging from 10–400 mg/kg, the most notable effect was a hypoglycemia at the two highest doses (Table I). This was significant at 400 mg/kg. The only significant increase in blood sugar was noted in the 4-hr sample at a dose of 50 mg/kg of triflocin. However, throughout the study increasing blood glucose levels in many groups were noted at the later time intervals. The possibility that this trend was the result of repeated bleeding of the animal was checked in a group of rats by omitting the earlier samples and taking the first sample at 4 hr after drug administration. In these experiments, the increasing blood glucose levels were absent and there was no significant hyperglycemia at any dose of drug.

Oral doses of triflocin did not alter the resting blood glucose levels in dogs. One hour after a single dose of from 10–200 mg/kg, the highest average blood glucose level for any treated group was 76 mg/100 ml. Control animals had an average blood glucose level of 72 mg/100 ml for the same period. The difference was not significant. Two and six hours after drug administration, the highest blood glucose for any treated group was 72 and 70 mg/100 ml, respectively. The blood glucose for the equivalent control group was identical in both periods. In contrast to this lack of effect on blood glucose, the diuretic effect of orally administered triflocin in dogs is quite apparent at 5 mg/kg and reaches a maximum at 80 mg/kg (8).

The effects of single oral doses of triflocin administered 2 hr before the glucose load on the oral glucose tolerance test in rats are shown in Table II A. The only significant elevation of the blood glucose above control occurred in the latter part of the glucose tolerance curve after a dose of 400 mg/kg of triflocin. In earlier portions of the curve at the two largest doses of triflocin (100 and 400 mg/kg) as well as at two points in the curve after 50 mg/kg triflocin the blood glucose was significantly lower in treated than in control animals. Note that with the two highest doses of triflocin the peak level after glucose loading is lower in the treated ani-

TABLE I. The Effect of a Single Oral Dose of Triflocin on Blood Sugar in Nonfasted Rats.[a]

Drug	Dose (mg/kg)	No. of animals	Blood sugar (mg/100 ml ± SE)				
			Hours after drug administration				
			0	1	2	4	6
Control	—	44	107 ± 2	107 ± 2	109 ± 2	112 ± 2	116 ± 2
Triflocin	10	8	103 ± 2	105 ± 2	108 ± 3	106 ± 2	105 ± 1[b]
	25	7	106 ± 3	100 ± 2	113 ± 2	111 ± 3	107 ± 3
	50	20	108 ± 2	111 ± 2	114 ± 2	120 ± 2[c]	122 ± 2
	100	17	107 ± 2	109 ± 2	109 ± 3	113 ± 3	114 ± 3
	200	15	106 ± 2	107 ± 2	102 ± 2	107 ± 3	111 ± 4
	400	26	108 ± 2	102 ± 2	97 ± 2[b]	103 ± 2[b]	109 ± 2[b]

[a] Male Wistar rats weighing 230–260 g were used. Triflocin was administered by gavage in 2 ml/kg of 2% starch. Control animals received only the vehicle.

[b] Significant hypoglycemia ($p < .05$) compared to the control group for the same time interval.

[c] Significant hyperglycemia ($p < .05$) compared to the control group for the same time interval.

TABLE II. Effect of Triflocin on Glucose Tolerance Tests in Fasted Rats.[a]

Drug	Dose (mg/kg)	No. of animals	Blood sugar (mg/100 ml ± SE) 0[b]	½	1	1½	2	3	4
A Effect of a single oral dose 2 hr before glucose load									
Control	—	24	72 ± 2	139 ± 4	130 ± 3	101 ± 3	96 ± 3	91 ± 2	100 ± 2
Triflocin	10	13	73 ± 2	150 ± 7	140 ± 4	111 ± 4	99 ± 3	95 ± 4	101 ± 3
	25	3	69 ± 1	135 ±14	124 ±12	100 ± 9	97 ± 2	97 ±13	93 ±10
	50	12	66 ± 2[d]	149 ± 6	128 ± 3	105 ± 2	97 ± 2	88 ± 3	91 ± 2[d]
	100	8	71 ± 4	118 ± 7[d]	115 ± 7[d]	100 ± 4	92 ± 7	86 ± 7	96 ± 3
	400	8	71 ± 4	123 ± 7[d]	125 ± 6	124 ± 8[e]	121 ±10[e]	115 ±11[e]	113 ±10
B Effect of a single sc dose 2 hr before glucose load									
Control	—	8	72 ± 1	130 ± 2	126 ± 5	110 ± 5	100 ± 6	97 ± 6	96 ± 6
Triflocin[c]	100	7	65 ± 2[d]	126 ± 4	121 ± 3	105 ± 3	97 ± 4	85 ± 3	90 ± 4
	400	8	69 ± 4	110 ± 5[d]	113 ± 6	120 ± 5	118 ± 6[e]	106 ± 5	106 ± 6
C Effect of a single sc dose just prior to glucose load									
Control	—	8	74 ± 2	149 ± 8	152 ± 4	117 ± 5	96 ± 1	—	—
Triflocin[c]	100	7	71 ± 2	134 ± 7	125 ± 9[d]	110 ± 7	99 ± 5	—	—
	400	7	70 ± 2	132 ± 9	110 ± 8[d]	93 ± 6[d]	104 ± 6	—	—

[a] Male Wistar rats weighing 200–230 g were used. Animals were starved 18 hr prior to the experiment but had water *ad libitum*.

[b] Preload (0) blood sample was obtained immediately before glucose loading.

[c] Triflocin was administered as its soluble sodium salt.

[d] Significant hypoglycemia ($p < .05$) compared to the control group for the same time interval.

[e] Significant hyperglycemia ($p < .05$) compared to the control group for the same time interval.

mals and the return to preloading glucose levels is slower in the group of animals that were given 400 mg/kg of triflocin.

Similar patterns were obtained when triflocin was given sc 2 hr before glucose rather than orally (Table II B). At doses of 100 and 400 mg/kg, triflocin produced some hypoglycemia. The only significant elevation in blood glucose was in the 2-hr period after a dose of 400 mg/kg. The hypoglycemic effect of sc administered triflocin was even more apparent when the drug was given immediately before the glucose load (Table II C). Within 2 hr there was no significant hyperglycemia.

The effects of a single oral dose of triflocin on intravenous glucose tolerance are shown in Table III. Doses below 400 mg/kg did not significantly alter the curve. However, 400 mg/kg of triflocin produced a hyperglycemic effect that was significant from 30 min to 2 hr after glucose loading. In no instance was there a significant hypoglycemic effect. Furosemide, at doses of 20 and 50 mg/kg, produced a marked hyperglycemia that was evident 2 min after glucose loading and was dose related. At the highest dose of furosemide, even the preload blood glucose was significantly elevated.

Effects of repeated doses of triflocin on glucose tolerance in rats. The effects of repeated daily doses of triflocin for 15 consecutive days prior to and including the day of the experiment are shown in Table IV. The final dose was given 2 hr before glucose loading. One group of animals was given furosemide, 20 mg/kg/day for comparison. Whereas furosemide caused a significant elevation of the peak blood glucose level (1/2 hr sample), the only significant elevation with triflocin was in the 2 hr sample after 400 mg/kg. Thus, as in the previous series of experiments, the only hyperglycemic effect of triflocin was some delay in the descending portion of the glucose tolerance curve toward normal. As was true after a single dose of triflocin, some of the earlier samples, including the peak glucose level, were significantly lower than the corresponding control samples.

TABLE III. The Effect of a Single Oral Dose of Triflocin or Furosemide on the Intravenous Glucose Tolerance Test in Fasted Rats.[a]

Drug	Dose (mg/kg)	No. of animals	Blood sugar (mg/100 ml ± SE)					
			Minutes after glucose load					
			0[b]	2	15	30	60	120
Control	—	15	68 ± 2	139 ± 3	121 ± 2	103 ± 3	88 ± 3	84 ± 3
Triflocin[c]	10	7	63 ± 2	138 ± 5	118 ± 3	104 ± 4	88 ± 4	90 ± 3
	100	10	66 ± 2	141 ± 4	120 ± 3	99 ± 3	85 ± 3	78 ± 4
	400	12	64 ± 2	144 ± 3	127 ± 3	115 ± 4[d]	109 ± 6[d]	95 ± 4[d]
Furosemide	20	6	67 ± 2	153 ± 3[d]	133 ± 6[d]	116 ± 6[d]	104 ± 5[d]	100 ± 6[d]
	50	12	74 ± 2[d]	158 ± 5[d]	137 ± 5[d]	122 ± 4[d]	112 ± 5[d]	104 ± 6[d]

[a] Male Wistar rats weighing 200–230 g were used. The animals were starved for 18 hr prior to the experiment but had water *ad libitum*.

[b] The preload (0) blood sample was obtained immediately before glucose loading.

[c] Drugs were administered 2 hr before the glucose load.

[d] Significant hyperglycemia ($p < .05$) compared to the control group for the same time interval.

Discussion. Triflocin, in single or repeated oral doses, did not produce hyperglycemia in rats (Tables I, IV) or dogs. Instead, hypoglycemia was a more common occurrence. However, some hyperglycemic tendency became apparent at high doses in glucose-loaded rats (Tables IIA, IV). In these animals the peak blood glucose level was significantly decreased in the treated animals but the return toward initial levels was delayed (Table IIA). These results seem to indicate a dual effect of triflocin, one on glucose absorption from the gastrointestinal tract and one on the metabolism and/or utilization of glucose. The hypoglycemia produced by large doses in nonfasted animals and the decrease in peak blood glucose levels in glucose-loaded animals receiving large doses of triflocin may have resulted from interference with the absorption of glucose from the gastrointestinal tract. This possibility is supported by the observation that when the glucose load

TABLE IV. The Effect of Repeated Daily Oral Doses of Triflocin or Furosemide for 15 Consecutive Days on the Oral Glucose Tolerance Test in Rats.[a]

Drug	Dose (mg/kg)	No. of animals	Blood sugar (mg/100 ml ± SE)				
			Hours after glucose load				
			0	½	1	1½	2
Control	—	49	81 ± 1	121 ± 2	125 ± 2	113 ± 2	104 ± 2
Triflocin	100	38	77 ± 1[b]	115 ± 2[b]	113 ± 3[b]	108 ± 3	105 ± 3
	400	24	74 ± 1[b]	116 ± 4	118 ± 3	119 ± 3	114 ± 4[c]
Furosemide	20	25	80 ± 1	137 ± 3[c]	132 ± 2	117 ± 3	107 ± 4

[a] Male Wistar rats were used. They weighed 200–230 g at the time of the glucose tolerance test. The animals were starved for 18 hr prior to the experiment but had water *ad libitum*. The final dose of drug was given 2 hr before glucose loading and the "O" blood sample was obtained immediately before glucose loading.

[b] Significant hypoglycemia ($p < .05$) compared to the control group for the same time interval.

[c] Significant hyperglycemia ($p < .05$) compared to the control group for the same time interval.

was administered intravenously, thus bypassing gastrointestinal absorption problems, 400 mg/kg of triflocin produced only a slight hyperglycemia (Table III). This observation also makes the possibility unlikely that the hypoglycemia produced by triflocin results from stimulation of insulin release, a mechanism that may account for the profound hypoglycemia produced by ethacrynic acid in a few patients (12). In addition, periodic sampling of the urine in each type of experiment failed to reveal any glucose in the urine.[3] Thus, the hypoglycemia after triflocin cannot be attributed to interference with renal reabsorptive processes and consequent urinary excretion of glucose. However, alteration of the gastrointestinal absorption of glucose by large doses of triflocin is not due to a local effect. The sc administration of triflocin had essentially the same effect on the glucose tolerance curve as orally administered triflocin (Tables IIB, IIC). Thus, triflocin appears to systemically affect the movement of glucose across the intestinal wall.

The extent to which triflocin changes glucose metabolism or utilization can best be assessed by comparison of the effect of a large dose on the iv glucose tolerance pattern with the effect of furosemide on this pattern (Table III). Although the diuretic potency of furosemide is five-ten times as great as that of triflocin on a milligram basis, it can be seen that the ability of furosemide to elevate the glucose tolerance curve was greater than that of triflocin even at equivalent diuretic doses. Thus, although triflocin and furosemide have nearly the same diuretic efficacy in rats and dogs, the present study indicates that triflocin has less tendency to produce hyperglycemia in these species.

[3] Absence of urinary glucose was confirmed by the use of Clinistix (Ames Co., Inc.).

Summary. The data obtained in the present study indicate that triflocin, a new diuretic, has no propensity for evoking hyperglycemia. In fact, it is so weakly hyperglycemic that special conditions, such as glucose loading, must be invoked before an increased blood glucose can be demonstrated. A greater tendency is for the drug to produce hypoglycemia, presumably by interference with the gastrointestinal absorption of glucose.

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1. Wales, J. K., Grant, A., and Wolff, F. W., J. Pharmacol. Exptl. Therap. **159**, 229 (1968).

2. Cattell, W. R., Practitioner **190**, 794 (1963).

3. Tabachnick, I. I. A., Gulbenkian, A., and Yannell, A., Life Sci. **4**, 1931 (1965).

4. Weller, J. M. and Borondy, M., Metabolism **16**, 532 (1967).

5. Foy, J. M., Life Sci. **6**, 897 (1967).

6. Cummings, J. R., Ronsberg, M. A., Stokey, E. H., and Gussin, R. Z., Pharmacologist **10**, 162 (1968).

7. Gussin, R. Z., Stokey, E. H., Ronsberg, M. A., and Cummings, J. R., Pharmacologist **10**, 163 (1968).

8. Gussin, R. Z., Cummings, J. R., Stokey, E. H., and Ronsberg, M. A., J. Pharmacol. Exptl. Therap., **167**, 194 (1969).

9. Riley, V., Proc. Soc. Exptl. Biol. Med. **104**, 751 (1960).

10. Hoffman, W. S., J. Biol. Chem. **120**, 51 (1937).

11. Snedecor, G. W., "Statistical Methods," 4th ed., Iowa State College Press, Ames, Iowa (1956).

12. Maher, J. F. and Schreiner, G. E., Ann. Internal Med. **62**, 15 (1965).

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