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10. The column, equilibrated with 10 m*M* potassium phosphate buffer (*p*H 6.2) containing 2 m*M* mercaptoethanol, was eluted with 0.5*M* NaCl in the same buffer.
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Inhibition of Catecholamine Release by Tolbutamide and Other Sulfonylureas

Abstract. *Tolbutamide and other sulfonylureas inhibited spontaneous and nicotine-induced release of catecholamines from the perfused cat adrenal gland and nicotine-induced release of [³H]norepinephrine from isolated guinea pig hearts. Of the sulfonylureas tested, the order of potency of this inhibitory effect paralleled the hypoglycemic action. These results raise the possibility that the inhibition of the sympathoadrenal system may contribute in part to the hypoglycemic action of sulfonylureas.*

Sulfonylureas are oral hypoglycemic agents which have been utilized extensively in the treatment of adult-onset diabetes mellitus. The primary action of this group of agents has been attributed to the direct stimulation of pancreatic beta cells to release insulin (*1*). However, the poor correlation between their insulin-releasing effect and diabetic control has led to speculation of extrapancreatic actions of sulfonylureas (*2*). Herein we report an effect of tolbutamide and other sulfonylureas on the spontaneous and nicotine-induced release of catecholamines from isolated cat adrenal glands (*3*) and on nicotine-induced release of [³H]norepinephrine ([³H]NE) from isolated guinea pig hearts (*4*). These observations suggest that sulfonylureas have a direct inhibitory action on the sympathoadrenal system.

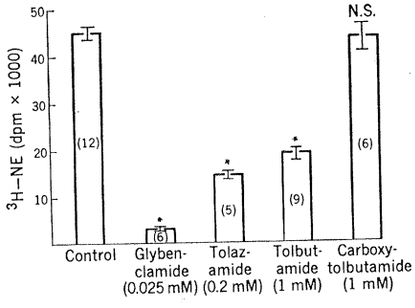

Fig. 1. The effects of four sulfonylureas on the release of [³H]NE induced by nicotine in isolated guinea pig hearts, measured in disintegrations per minute. Release of [³H] was stimulated by a single injection of nicotine. Five minutes before and after injection, one of the sulfonylureas or the vehicle used to dissolve sulfonylureas (control) was present. Asterisk denotes difference from control is significant at $P < .001$. *N.S.*, not significant.

Isolated cat adrenal glands were retrogradely perfused with phosphate-buffered Locke's solution, and the adrenal catecholamine secretion was monitored by a modification of the procedure of Robinson and Watts (*5*). This experimental procedure has been reported in detail previously (*6*). The effect of tolbutamide on the spontaneous secretion of catecholamines was observed by perfusing the glands with Locke's solution containing tolbutamide (0.1, 0.3, or 1 m*M*) for 5 minutes. To study the effect of sulfonylureas on the nicotine-induced release of catecholamines, each gland was stimulated twice by perfusion with nicotine ($10^{-6}M$) for 1 minute. Five minutes before and 5 minutes after the first stimulation by nicotine, the adrenal was perfused with either tolbutamide (0.3 or 1 m*M*) or tolazamide (0.2 m*M*). The administration of nicotine was repeated 30 minutes after termination of the perfusion with sulfonylureas and served as a control response.

Endogenous norepinephrine stores in isolated guinea pig hearts were prelabeled with [³H]NE. Hearts were stimuated to release [³H]NE by single injections of nicotine (4×10^{-7} mole) 20 minutes after [³H]NE labeling. The perfusate effluents from the hearts were continuously collected and analyzed for [³H]NE by liquid scintillation spectrometry (*7*). To study the effect of sulfonylureas on nicotine-induced release of myocardial [³H]NE, sulfonylureas (tolbutamide, 1 m*M*; carboxytolbutamide, 1 m*M*; tolazamide, 0.2 m*M*; glybenclamide, 0.025 m*M*) were individually added to the perfusion fluid from 5 minutes before to 5 minutes after the injection of nicotine.

The spontaneous output of catecholamines from the cat adrenal gland usually became stable 1 hour after the initiation of perfusion with Locke's solution. Tolbutamide (0.3 or 1 m*M*) caused a decline in spontaneous catecholamine output (see Table 1). This inhibitory effect of tolbutamide was reversible. Nicotine-induced release of adrenal catecholamines was also suppressed by tolbutamide. When adrenal glands were stimulated consecutively at 30-minute intervals by the same dose of nicotine ($10^{-6}M$ for 1 minute), the response to the second stimulation was only 60 to 70 percent of the initial response (data not shown). When tolbutamide (0.3 or 1 m*M*) was present only during the initial exposure to nicotine, the second response to nicotine was then greater than the first. Tolazamide (0.2 m*M*) exerted a similar inhibitory action (see Table 1).

Nicotine-induced release of [³H]NE from the isolated guinea pig hearts was also inhibited by several sulfonylureas (tolbutamide, tolazamide, and glybenclamide). The order of potency in inhibiting catecholamine release by these

Table 1. Effect of tolbutamide and tolazamide on spontaneous and nicotine-induced release of total catecholamines from isolated cat adrenal glands. Each gland served as its own control. The data are expressed as percent of control. The number of glands studied is indicated in parentheses after the mean ± S.E.M.

Drugs (m*M*)	Catecholamines released (% of control)	
	Spontaneous*	Nicotine-induced†
Tolbutamide		
0.1	95 ± 2 (3)	
0.3	72 ± 6 (5)‡	77 ± 5 (4)‡
1.0	62 ± 8 (5)‡	56 ± 10 (7)‡
Tolazamide		
0.2		51 ± 7 (3)‡

* [Rate of spontaneous output in the presence of sulfonylurea (ng/min) divided by the rate of spontaneous output in the absence of sulfonylurea (ng/min)] × 100. Control spontaneous output was 230 ± 30 ng/min. † [Nicotine-induced release in the presence of sulfonylurea (ng) divided by nicotine-induced release in the absence of sulfonylurea (ng) × 100. Control nicotine-induced release was 12,210 ± 1,330 ng. ‡ Signifiant at $P < .01$.

sulfonylureas was glybenclamide > tolazamide > tolbutamide (Fig. 1). Carboxytolbutamide, a major metabolite of tolbutamide without hypoglycemic action (*8*), showed no inhibitory effect on induced [^{3}H]NE release.

In vivo studies have led to the postulate that sulfonylureas may possess a direct stimulatory action on the adrenal medulla (*9*). Since epinephrine release is increased by insulin-induced hypoglycemia (*10, 11*), the previous in vivo observations with sulfonylureas may be an indirect result of the hypoglycemia following insulin release. The present in vitro studies demonstrate that sulfonylureas act directly to inhibit the release of catecholamine from the feline adrenal medulla and from the adrenergic nerve terminals in guinea pig hearts. This effect of sulfonylureas was observed when catecholamine secretion was stimulated by nicotine or other secretagogues such as glucagon or KCl (*3, 4*). Pittman and Hazelwood selected doses of insulin and tolbutamide which caused similar degrees of hypoglycemia in chickens and found elevated plasma epinephrine levels only in those animals in which the hypoglycemia was induced by insulin (*11*). This absence of epinephrine release in response to tolbutamide-induced hypoglycemia suggests that this drug probably inhibits adrenal catecholamine release in intact animals.

In general the metabolic effects of catecholamines oppose the actions of insulin (*12*). Physiological concentrations of catecholamines are known to inhibit insulin release (*13*). It was interesting to note that for the two sulfonylureas tested in cat adrenal glands and for the four tested in guinea pig hearts, the order of potency in inhibiting catecholamine release paralleled their hypoglycemic potency (*1, 9*).

Results from the present experiments raise the possibility that this extrapancreatic action on the sympathoadrenal system may contribute in part to the hypoglycemic effect of sulfonylureas.

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Batrachotoxin Block of Fast Axoplasmic Transport in Mammalian Nerve Fibers

Abstract. *Batrachotoxin (BTX) irreversibly blocks fast axoplasmic transport in nerve in concentrations as low as 0.2 micromolar. The action of BTX was studied in cat sciatic nerves in vitro by measuring the rate of the crest outflow after injection of the L7 dorsal root ganglion with [^{3}H]leucine. Tetrodotoxin, which in itself does not affect fast axoplasmic transport, inhibited the blocking action of BTX. Unlike the BTX block of nerve and muscle membrane excitability brought about through increased permeability to sodium ion, the BTX block of fast axoplasmic transport occurs with or without sodium ion in the medium. High concentrations of calcium ion protected against the blocking action of BTX, while magnesium ion did not. An action of BTX on the transport mechanism inside the fibers was indicated by the small reduction of adenosine triphosphate plus creatine phosphate, which in itself did not account for the block of axoplasmic transport.*

The lack of a direct dependence between fast axoplasmic transport (*1*) and membrane excitability in nerve fibers was shown by the observation that either tetrodotoxin (TTX) or procaine, in concentrations adequate to block excitability failed to affect fast axoplasmic transport (*2*). Furthermore, the replacement of Na^+ in the Ringer solution for in vitro studies with an equivalent amount of K^+, and the resulting depolarization, had no effect on axoplasmic transport; nor, for that matter, did removal of all ions by the replacement of Ringer solution with an isotonic sucrose solution (*3*). On the other hand, a membrane effect was indicated when nerves were electrically stimulated to elicit maximal alpha responses at 100 pulses per second for 3 to 5 hours; in this case, a small but definite 10 percent decrease in axoplasmic transport was found (*4*). Transport was shown to cease temporarily (for about 0.75 hour), with recovery in the face of maintained stimulation (*5*). This block was not due to a reduction in the level of adenosine triphosphate (ATP) or creatine phosphate (CP) resulting from the increased activity (*5*). To test the possibility that an increased influx of Na^+ led to the temporary block of transport, we studied the effect of batrachotoxin (BTX) on fast axoplasmic transport; BTX is thought to cause block of membrane excitability through increased Na^+ permeability (*6*).

As usual in our in vitro studies of axoplasmic transport, the L7 dorsal root ganglia of cats were injected with [^{3}H]leucine, and 2 hours were allowed for the downflow of the labeled com-

Table 1. Blocking time of axoplasmic transport with BTX. Nerves were removed 2 hours after injection and placed in vitro for 4 hours or longer. The blocking was determined by the position of the front of the crest compared with control rate of 17 mm/hour; the rate is linear (*7*). Abbreviations: *N*, number of experiments; NB, no block within the downflow period; 0, immediate block within 10 minutes.

Batrachotoxin (μM)	*N*	Time to block (hours)
0.02	1	NB
0.05	5	NB, NB, NB, 2.35, 3.53
0.1	2	NB, 2.35
0.2	1	2.94
0.4	5	1.93 ± 0.15*
1.0	7	1.60 ± 0.28*
2.0	3	0.84 ± 0.28*
3.0	1	0.29
3.7	1	0.94
4.6	1	0.58
9.3	1	0
93.0	1	0

* Mean ± standard error.