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.

ponents in the sensory fibers of the sciatic nerves in vivo (*7*). The sciatic nerves were then rapidly removed and placed in 25-ml flasks that contained 10 to 20 ml of isotonic NaCl or sucrose kept at 38°C and vigorously bubbled with 95 percent O_2 plus 5 percent CO_2. The nerves remained in the flasks for a further period of in vitro transport, which typically lasted 4 to 6 hours.

When added to the in vitro medium, BTX produced a block of fast axoplasmic transport corresponding to its potency in blocking excitation of nerve and muscle membranes. The block of transport was shown by the failure of the crest of labeled activity to move down the nerves to the distance expected for an untreated nerve (Fig. 1). The control nerve in this example shows the usual pattern and rate of fast axoplasmic transport (*7*); the rate (410 mm/day) was determined by the distance to which the crest had advanced in the total time of downflow, in this case 5.5 hours (2 hours in vivo plus 3.5 hours in vitro). The position of the front of the crest in the nerve treated with BTX did not advance much beyond that occurring in vivo and fell far short of the control distance. The time at which block occurred was reduced with higher concentrations of BTX in the medium (Table 1). Even at a concentration as low as 0.05 μM, a small blocking effect was seen in one sample; more definite block was seen with concentrations of BTX 0.2 μM and greater. The blocking effect of BTX was irreversible after a 1-hour exposure.

The vinca alkaloids vinblastine and vincristine, which are considered to be potent agents in blocking fast axoplasmic transport, showed a comparable block at 1 to 2 mM in the same in vitro system (*8*); BTX is thus 5,000 to 10,000 times more potent in this respect than the vinca alkaloids.

Tetrodotoxin, which blocks the action of BTX on membrane excitability (*9*), also interfered with the blocking action of BTX on fast axoplasmic transport. Nerves that were first treated with TTX and showed no action potentials when stimulated in a chamber (*4*) were exposed as usual to BTX in vitro. In these TTX-treated nerves, BTX did not block fast axoplasmic transport.

If BTX blocks axoplasmic transport as a result of an increased entry of Na^+ into the fibers, it should be ineffective in a Na^+-deficient medium, as demonstrated by its effects on nerve and muscle excitability (*9*). However, the block of fast axoplasmic transport produced by BTX was just as effective in an isotonic sucrose medium without Na^+ as it was in isotonic NaCl (Fig. 2). Some other mechanism of action must therefore be invoked to account for the BTX block of axoplasmic transport.

In the model proposed for fast axoplasmic transport, the Mg^{2+}, Ca^{2+}-adenosine triphosphatase in nerve (*10*) is considered to utilize ATP by means of cross-bridges acting between the transport filaments and microtubules (*1*). Various components are bound to the transport filaments and thus moved down the nerve fibers. The possibility that BTX could reduce the supply of ATP needed to maintain fast axoplasmic transport was tested by measuring the combined concentration of ATP and CP [high energy phosphate ($\sim$ P)] representing energy available to the transport mechanism (*11*). Batrachotoxin in concentrations effective in blocking fast axoplasmic transport (0.4 to 5 μM) had a small effect on the concentration of $\sim$ P, reducing it to 12 percent below the control value of 1.40 μM/g. This decrease was much smaller than the fall of 0.6 μM/g associated with anoxia-induced block of fast axoplasmic transport (*11*). With 10 μM BTX, $\sim$ P was markedly reduced, a finding similar to that reported for BTX in muscle (*12*).

A possible relation between Na^+ entry and Ca^{2+} regulation in the fiber (*13*) suggested that BTX could have its effect on the transport filament mechanism through an altered Ca^{2+}

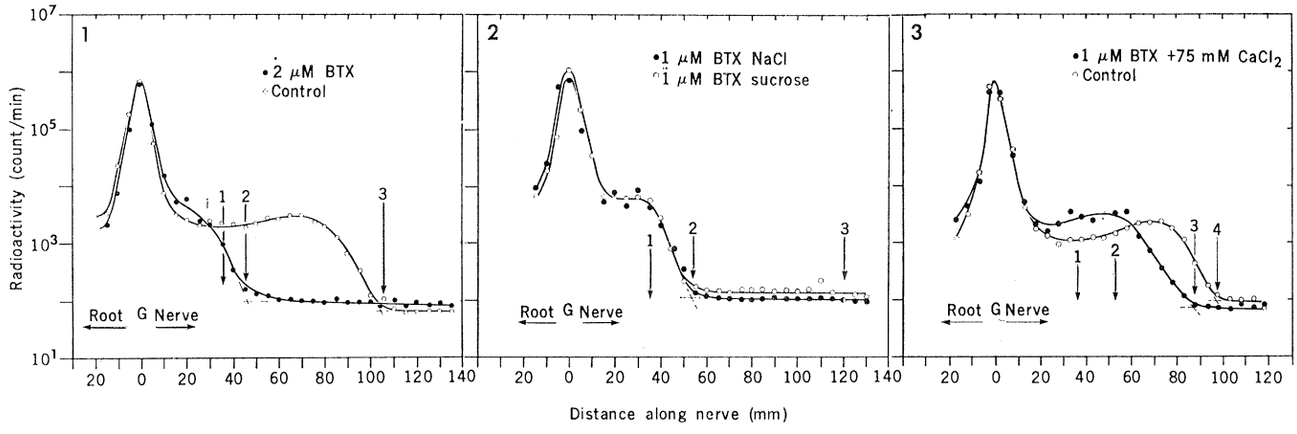

Fig. 1. Block of axoplasmic transport in vitro by BTX as shown by the pattern of outflow of radioactivity in sciatic nerve fibers after [^{3}H]leucine injection of the L7 dorsal root ganglion of the cat. The patterns were obtained after 2 hours of transport in vivo and 3.5 hours in vitro. In the control nerve, a large pool of activity remains in the region of the injected ganglion (*G*), with a lower plateau rising to a distal crest and falling to baseline levels (arrow 3). The position to which the front of the crest is calculated to have moved after 2 hours of in vivo downflow at the usual rate of 410 mm/day is shown by arrow 1. With 2 μM BTX present in vitro, the front of the crest (arrow 2) has advanced only slightly beyond arrow 1; this advance corresponds to an additional 0.5 to 0.75 hour of transport. Each point represents a 5-mm segment of nerve solubilized and individually counted in a Packard 3310 spectrometer. Fig. 2. Block of transport by 1 μM BTX with isotonic NaCl or isotonic sucrose present in the medium in vitro. The block produced with BTX (arrow 2) represents an additional downflow in vitro of no more than 0.5 to 0.75 hour beyond the in vivo transport (arrow 1). The distance to which the crest would normally move without the block is shown by arrow 3. Fig. 3. Reduction by $CaCl_2$ of the degree of block produced by 1 μM BTX (Fig. 2). With 75 mM $CaCl_2$ in the in vitro medium, the crest position (arrow 3) has moved further down the nerve, closer to the position of the crest of the control nerve (arrow 4). Arrow 1 shows the downflow in vivo, and arrow 2 indicates the downflow expected with this concentration of BTX without added Ca^{2+}.

level in the nerve fibers. Nerves placed in media containing 35 to 125 m*M* Ca^{2+} were protected against an amount of BTX adequate to block fast axoplasmic transport (Fig. 3). Similarly high concentrations of Mg^{2+} did not interfere with the BTX block of fast axoplasmic transport, a result suggesting some specific role for Ca^{2+}.

These studies do not discriminate between an effect of BTX on some membrane component to which Ca^{2+} is also bound (*14*) and an action of BTX (with which Ca^{2+} interferes) on the axoplasmic transport mechanism within the nerve fiber. An action of BTX within the fiber is indicated by the small decreases of ~P levels at BTX concentrations effective in blocking transport, and a block of transport on the basis of a direct action of BTX on membrane excitability is excluded in that other agents that block membrane excitability do not block axoplasmic transport (*2, 3*). However, an indirect effect of BTX on the membrane, in turn affecting the axoplasmic transport mechanism, also remains a possibility. Further studies should help resolve this point.

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Homologous Cysteine-Containing Sequences in Tryptophanyl-tRNA Synthetases from Escherichia coli and Human Placentas

Abstract. *The sequence Leu-Ala-Cys-Gly-Ile-Asx-Glx in a nonapeptide isolated from the tryptophanyl-tRNA synthetase of* Escherichia coli *B is homologous to the sequence Ile-Ala-Cys-Gly-Phe-Asx-Asx in a decapeptide isolated from the tryptophanyl-tRNA synthetase of human placenta. So far no homologies have been found between cysteine-containing peptides of aminoacyl-tRNA synthetases with different amino acid specificities.*

The aminoacyl-tRNA synthetases comprise a family of enzymes required to recognize specific amino acids and cognate transfer RNA's (tRNA's). The tRNA's are gene products uniform in size, in secondary structure, and probably in tertiary structure (*1*) over an evolutionary range from bacteria to mammals. The necessary coordinate evolution of a synthetase and its tRNA substrate should cause synthetases evolved from a common ancestor to preserve essential structural features. Although the aminoacyl-tRNA synthetases present an array of quaternary structures (α_1, α_2, α_4, $\alpha\beta$, $\alpha_2\beta_2$) with protomer molecular weights ranging from 33,000 to 114,000 (*2*), those specific for the same amino acid generally have similar protomer sizes and quaternary structures.

The tryptophanyl-tRNA synthetases have been purified to homogeneity from *Bacillus stearothermophilus* (*3*), *Escherichia coli* (*4*), yeast (*5*), beef pancreas (*6*), water buffalo brain (*7*), and human placenta (*8*). With one possible exception (*7*), they consist of two identical subunits, which range from a low molecular weight of 35,000 for the enzyme from *B. stearothermophilus* to a higher molecular weight of 58,000 for both the bovine (*6*) and human enzymes (*8*). Not surprisingly, the bovine and human tryptophanyl-tRNA synthetases are also similar in amino acid compositions, tryptic peptide maps (*9*), and interactions with tryptophan tRNA ($tRNA^{Trp}$) of yeast and *E. coli* (*10*), whereas the *E. coli* tryptophanyl-tRNA synthetase, molecular weight 74,000, has a different amino acid composition (*4*) and pattern of interaction with $tRNA^{Trp}$ of yeast and of *E. coli* (*8*). However, the bovine enzyme can be cleaved to an active α_2 structure of molecular weight 82,000 (*11*), which approaches more closely the composition of the *E. coli* enzyme. Therefore, it is likely that a portion of the bovine (or human) enzyme resembles the *E. coli* enzyme more closely than is ap-

Table 1. Recoveries, NH_2-terminal amino acids, and compositions of three thiol peptides purified from *Escherichia coli* and two thiol peptides from human placental tryptophanyl-tRNA synthetases. The values in parentheses are the whole numbers assigned.

Amino acid residue	Composition (moles of amino acid per mole of peptide)				
	Escherichia coli			Human placenta	
	IIIA2 191 nmole*	IA91 428 nmole*	IA131 126 nmole*	TC2 180 nmole†	TC3 90 nmole†
Lysine	0.0	1.0 (1)	0.2	1.2 (1)	1.1 (1)
Histidine	0.2	0.1	0.8 (1)	0.0	0.0
Arginine	0.9 (1)	0.1	0.9 (1)	0.2	0.2
S-Carboxymethyl-cysteine	0.5 (1)	0.8 (1)	0.7 (1)‡	0.4 (1)	0.4 (1)
Aspartic acid or asparagine	1.0 (1)	1.1 (1)	1.1 (1)	2.9 (3)‡	2.2 (2)
Threonine	0.9 (1)	0.2	0.9 (1)	0.1	1.1 (1)
Serine	1.0 (1)	0.3	0.3	0.2	1.1 (1)
Glutamic acid or glutamine	1.2 (1)	1.2 (1)	1.1 (1)	0.2	0.3
Proline	0.0	1.0 (1)	0.0	0.0	0.0
Glycine	1.2 (1)	1.1 (1)	0.4	1.2 (1)	3.0 (3)‡
Alanine	1.0 (1)‡	0.9 (1)	1.0 (1)	1.2 (1)	0.2
Valine	0.2	0.3	1.6 (2)	0.1	0.2
Methionine	0.0	0.0	0.0	0.0	0.0
Isoleucine	0.2	0.9 (1)	1.4 (2)	2.1 (2)	2.0 (2)
Leucine	1.8 (2)	1.0 (1)‡	0.1	0.0	0.0
Tyrosine	1.5 (2)	0.0	0.0	0.0	0.0
Phenylalanine	0.8 (1)	0.1	0.0	1.0 (1)	2.0 (2)

* The recovery from 1350 nmole of peptide. † The recovery from 500 nmole of peptide. ‡ The amino acid at the NH_2-terminal of the peptide.