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ENDOTOXIN AND TRYPTOPHAN-INDUCED HYPOGLYCAEMIA IN RATS

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Abstract—Pretreatment of rats with increasing, but non-lethal, doses of endotoxin was associated with a parallel increase in sensitivity to induction of hypoglycaemia by tryptophan. Acutely streptozocin-diabetic animals became hypoglycaemic with endotoxin alone, and this was increased further by tryptophan. Variations in tryptophan sensitivity between rat populations cannot be explained by previous history of exposure to endotoxin. Endotoxin abolished the increase in tryptophan dioxygenase activity caused by triamcinolone, but not that caused by tryptophan. Triamcinolone was effective, however, when given together with tryptophan to endotoxin-treated rats. The activity of tryptophan dioxygenase *in vivo* and in liver cells *in vitro* is unchanged by exposure to endotoxin at 1 mg/kg body wt. Turnover studies indicated that hypoglycaemia resulted from inhibition of gluconeogenesis. There was no evidence to support a role for insulin in this process and results were consistent with an endotoxin-mediated hepatic insensitivity to glucagon. They also suggested that quinolinate, rather than 5-hydroxytryptamine, may be the intracellular agent responsible for inhibition of gluconeogenesis.

The shock syndrome following administration of endotoxin from Gram-negative bacteria is complex and involves many tissues and systems in the body [1, 2]. There is some disagreement as to which of the effects are primary, and resolution of this problem is complicated by the wide range of endotoxin preparations, experimental regimes and the innate responsiveness of several species and strains. There is, however, some consensus that exposure to endotoxin results in profound hypoglycaemia following a transitory hyperglycaemia [3, 4], and that this may be one of the crucial reactions in the development of the syndrome [5]. It has been claimed that endotoxic shock may be substantially alleviated by exogenous glucose in dogs [6, 7]. Nevertheless, experimental diabetes, in which blood glucose levels are maintained above normal values even in endotoxaemia, does not seem to confer any immunity [8–10]. These observations also do not support the proposition that endotoxin actions may be mediated, at least in part, through hyperinsulinaemia [11].

The decrease in blood glucose arises from a decline in gluconeogenesis [3, 8, 12, 13], although there is strong evidence favouring a smaller increase in peripheral glucose uptake [14, 15]. A component of the inhibition of glucose production is thought to be the block of the induction of phosphoenolpyruvate carboxykinase [GTP:oxaloacetate carboxylyase (transphosphorylating) EC 4.1.1.32] by glucocorticoids [16–18]. Although liver cells from endotoxin-treated rats show impaired gluconeogenesis, 'normal' cells do not respond when incubated with endotoxin *in vitro* [19, 20]. This may be attributed to a requirement for the formation of a mediator *in vivo*, a process probably involving the reticuloendothelial system [20–23].

Tryptophan is hypoglycaemic in rats under a number of conditions [2, 25], and it is known that this amino acid increased the morbidity associated with endotoxaemia [26–29]. The mechanism of this interaction is unclear, but 5-hydroxytryptamine has been proposed as an important factor ([28, 29]; see also ref. [24]).

Reports of tolerance to repetitive doses of endotoxin suggested to us that some of the variability in the responsiveness to tryptophan of apparently similar rat populations [25, 30] might be explicable in terms of differential exposure to bacterial infection. This is not an unreasonable suggestion, since endotoxin, or endotoxin 'mediators', are widely reported to inhibit the action of glucocorticoids [21, 23] which in turn, would produce the differential sensitivity to glucagon suggested by our laboratory as a possible explanation of tryptophan-sensitive and tryptophan-resistant rat populations [25]. The studies reported in this paper were designed to investigate both this possibility and further details of the interaction between endotoxin and tryptophan administration. The evidence does not provide unequivocal support for a role of 5-hydroxytryptamine or tryptamine in this system.

MATERIALS AND METHODS

The sources and treatment of animals and materials were as given in ref. [25]. Endotoxin,

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derived from *Salmonella typhimurium* by a phenolic extraction procedure, was from Sigma, Poole, U.K.; xylamidine tosylate was a gift from the Wellcome Research Laboratories, Beckenham, U.K.

All compounds for injection, other than tryptophan, were administered as solutions or suspensions in 0.9% (w/v) NaCl; control animals received vehicle alone.

Tryptophan dioxygenase (L-tryptophan:oxygen 2,3-oxidoreductase, EC 1.13.11.11) was assayed according to ref. [31]. Livers were removed, rinsed in 0.9% (w/v) NaCl, blotted, weighed and homogenized in 20 mM potassium phosphate/0.14 M KCl, pH 7.0, with a mechanical tissue disintegrator (Ultra-Turrax, Scientific Instruments, London, U.K.). Extracts were centrifuged at 38,000 *g* for 40 min at 0° before assay.

RESULTS AND DISCUSSION

Tryptophan is generally reported to be hypoglycaemic when administered by diverse routes to rats [24, 32–35]. There are, however, significant variations in the sensitivity to this amino acid, attributable both to nutritional and hormonal factors and also to differences, as yet undefined, between populations [24, 25, 30].

When rats from a population normally unresponsive to tryptophan were pre-treated with endotoxin, they became increasingly sensitive to the hypoglycaemic action of tryptophan as the dose of endotoxin was increased up to 1 mg/kg body wt (Fig. 1). The time-course of hypoglycaemia is similar to that found in other groups of rats and under other predisposing conditions [24, 25, 30].

Even at 2 mg/kg body wt endotoxin did not itself cause any decrease in blood glucose concn. Animals so treated all survived for at least 72 hr (as expected with the sublethal doses employed).

Similar rats rendered acutely-diabetic with streptozotocin did, in contrast, respond to endotoxin alone [plasma glucose concns falling from 36.5 ± 0.8 mM to 24.2 ± 1.1 mM (means ± S.E.; $n = 4$) in 3 hr] and this effect was increased by tryptophan given 16 hr later (Table 1), although the concn of glucose in plasma samples did not fall to values as low as those observed with non-diabetic controls. These results, which complement those of other workers [9, 10, 36], do not support the suggestion that the hypoglycaemia of endotoxicosis may be insulin-mediated [4, 11, 19, 37].

A second population of rats, which exhibited a marked hypoglycaemic response to tryptophan [25], were similarly apparently more sensitive to endotoxin. Sixteen hours after injection of endotoxin, plasma glucose concns were decreased by 50% (Table 2), although there was no further effect of tryptophan. When a further group of similar rats was pre-treated (to induce endotoxin resistance [1, 10, 26]) with a very low dose of endotoxin 7 days previously, the hypoglycaemic response, noted above, was abolished. These animals were, however, sensitive to tryptophan, which occasioned a 50% decrease in plasma glucose concns after 1.5 hr (Table 2).

These experiments were undertaken partly in an attempt to ascertain whether the differences in tryptophan sensitivity between rat populations [25] could be understood in terms of exposure to bacterial infection. Endotoxin is, however, unlikely to be an important factor, since exposure of tryptophan-sensitive rats to endotoxin does not decrease responsiveness to the amino acid, as might be predicted. Further, if tryptophan sensitivity were found only in endotoxin-exposed animals, one would have expected that the responsive group would be unaffected by the pretreatment regime of Table 2. However, in the light of the above results, the hypothesis that endotoxin administration may regulate the response to tryptophan (possibly via glucocorticoid activity) remains valid, and the mechanism whereby tryptophan elicits its hypoglycaemic response in endotoxin-treated rats was investigated further.

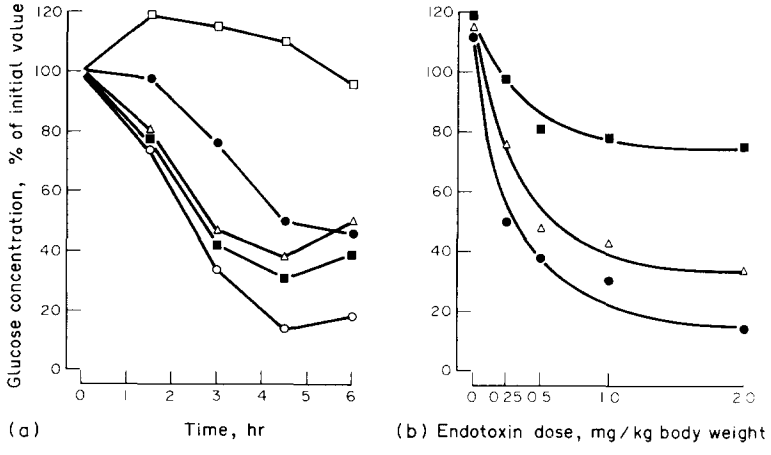

Fig. 1. Effect of tryptophan (750 mg/kg body wt, i.p.) on plasma glucose concns in 48 hr starved rats pretreated with endotoxin (16 hr previously, i.p.). The initial glucose concn was 5.9 ± 0.1 mM. Results are the means of eight observations; the S.E.s (all < 10%) have been omitted for clarity. (a) Time course: endotoxin dosage: (●) 0.25 mg/kg; (△) 0.5 mg/kg; (■) 1.0 mg/kg; (○) 2.0 mg/kg; (□) 0.9% NaCl (control). (b) Dose-response: (■) 1.5 hr; (△) 3 hr; (●) 4.5 hr after tryptophan loading.

Table 1. Effect of a tryptophan load on plasma glucose concns in acutely streptozotocin-diabetic rats treated with endotoxin*

Time (hr)	Diabetic [plasma glucose concn (mM)]		Endotoxin-treated diabetic [plasma glucose concn (mM)]	
	Control (n = 11)	Tryptophan (n = 12)	Control (n = 5)	Tryptophan (n = 5)
0	30.5 ± 1.9	—	13.2 ± 0.8	—
1.5	25.6 ± 1.1	20.7 ± 2.7	14.2 ± 0.7	7.5 ± 0.4***
3	24.9 ± 1.1	17.2 ± 2.0**	12.6 ± 0.6	4.3 ± 0.5***
4.5	24.2 ± 1.1	15.8 ± 1.3***	14.3 ± 0.6	4.7 ± 0.6***
6	23.9 ± 1.0	12.8 ± 1.1***	14.6 ± 0.6	3.7 ± 0.4***

* Streptozotocin (60 mg/kg body wt, i.v.) was given 48 hr, and endotoxin (1 mg/kg body wt, i.p.) 16 hr, before tryptophan. Tryptophan (750 mg/kg body wt, i.p.) was given at zero time. Results are means ± S.E. P (vs corresponding controls): ** < 0.01; *** < 0.001.

The sensitization, by endotoxin, of rats to tryptophan-induced hypoglycaemia could involve increased glucose utilization [4, 5, 9, 15], decreased glucose production [3, 8, 19, 20, 37], or a combination of both. Continuous infusion experiments with [2-^{3}H]glucose before and after administration of tryptophan showed that, under the conditions of these studies, virtually all of the decline in plasma glucose concn was accounted for by the decrease in glucose production (R_a; Fig. 2). Although there is a simultaneous small fall in R_d, the rate of glucose disposal, a proportion, at least, of this is predicted from the concn dependence of the various uptake processes; some of the fall in both R_a and R_d could also be attributed to a decrease in the amount of substrate cycling through glucose 6-phosphatase.

The lethal effects of endotoxin administration have been correlated with decreases in the activities and concns of a number of potentially rate-limiting enzymes [26, 38]. Tryptophan dioxygenase [tryptophan pyrrolase; L-tryptophan-oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11] activity increases immediately after endotoxin injection but decreases, to values less than those in controls, over a longer period [26, 39]. Animals are reported to be unaffected by tryptophan during the period of elevated tryptophan dioxygenase activity, but become sensitive as the enzyme content declines [29]. Glucocorticoids, which attenuate the response to endotoxin when both are injected simultaneously, are ineffective both in reversing the deleterious effects and in inducing enzyme synthesis when given after the toxin [26]. Table 3 shows the changes in tryptophan dioxygenase activity (which probably parallel changes in enzyme protein [40]) under various conditions. The enzyme increased 2.5–3-fold 3 hr after tryptophan injection in the control, triamcinolone- and endotoxin-treated animals. Triamcinolone was itself an effective inducer in controls, but had no effect in animals previously given endotoxin. When both triamcinolone and tryptophan were given to endotoxin-treated animals, however, the response was equivalent to that in controls under similar conditions, i.e. tryptophan had a 'permissive' effect for glucocorticoid activity. The observation that tryptophan itself was more effective in endotoxin-treated than in control animals may be related to an increase in the amount of circulating glucocorticoids in the former group [41].

Adrenalectomized rats were, as previously reported [26, 42], more sensitive to endotoxin; 75% mortality occurred within 16 hr of injection of 1 mg toxin/kg body wt. Triamcinolone given (as a single injection of 5 mg/kg body wt) to normal animals 1 hr before endotoxin attenuated, but did not abolish, the tryptophan-induced hypoglycaemia (results not shown; see also refs. [10, 23]) despite the high activities of tryptophan dioxygenase under these conditions.

Moon [29, 39] has suggested that tryptophan toxicity in endotoxin-treated animals is greatest when the activity of tryptophan dioxygenase is lowest.

Table 2. Effect of pretreatment with endotoxin on plasma glucose concns in 48 hr starved rats sensitive to tryptophan*

Time (hr)	Control [plasma glucose concn (mM)]		Endotoxin [plasma glucose concn (mM)]		Endotoxin-resistant [plasma glucose concn (mM)]	
	Control	Tryptophan	Control	Tryptophan	Control	Tryptophan
0	4.0 ± 0.2	—	2.0 ± 0.3	—	3.8 ± 0.1	—
1.5	3.6 ± 0.2	1.9 ± 0.2***	2.0 ± 0.5***	1.7 ± 0.1	4.0 ± 0.1	1.8 ± 0.1***
3	4.9 ± 0.2	2.2 ± 0.1***	2.0 ± 0.4***	2.0 ± 0.2	4.0 ± 0.1	1.9 ± 0.1***
4.5	4.4 ± 0.1	3.3 ± 0.1***	1.6 ± 0.4***	2.3 ± 0.3	4.0 ± 0.1	1.9 ± 0.3***

* Endotoxin (1 mg/kg body wt, i.p.) was given 16 hr before tryptophan (750 mg/kg body wt, i.p., zero time). Endotoxin resistance was induced by i.p. injection of endotoxin (100 μg/kg body wt) 7 days before food withdrawal. Results are means ± S.E. from six observations throughout. P (vs relevant controls): *** < 0.001.

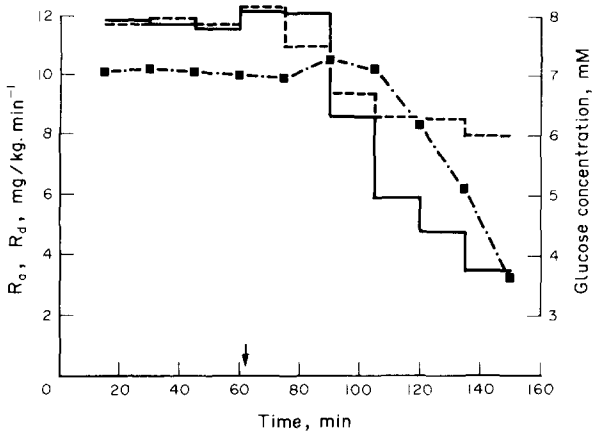

Fig. 2. Glucose turnover in 48 hr starved rats treated with endotoxin (1.0 mg/kg body wt, i.p.) 16 hr before injection of L-tryptophan (arrowed; 750 mg/kg body wt, i.p.). [2-^{3}H]Glucose was infused as previously described [25]. Controls, without tryptophan, yielded time-courses essentially parallel to the ordinate axis. This experiment is typical of three performed. (——) Rate of appearance of glucose, R_a; (- - -) rate of disappearance of glucose, R_d; (■) plasma glucose concn.

Under these circumstances, a loading dose of tryptophan would be oxidized less effectively with the result that more tryptophan would be diverted to 5-hydroxytryptamine synthesis, with consequent hypoglycaemia [24, 34, 35]. With the lower doses of endotoxin in these experiments, the effectiveness of tryptophan does not correlate, in the manner thus predicted, with the activity of the dioxygenase. It has been claimed, however, that endotoxin may, at least acutely, alter the amount of 'free' haem in the liver, and that this could, secondarily, increase the ratio of tryptophan dioxygenase holo- to apo-enzyme with consequent stimulation of activity *in vivo* [43, 44]. There might, therefore, be alterations in metabolic flux in the absence of changes in overall enzyme activity. We believe that this is unlikely to occur in the experiments described here. Firstly, determination of holo- to apo-enzyme activities in liver extracts from endotoxin-treated and control animals gave a ratio of approx. 70:30 in both groups

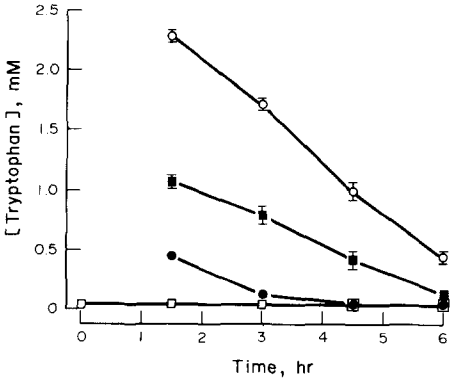

Fig. 3. Effect of tryptophan loading on plasma tryptophan concns in 48 hr starved rats treated with endotoxin (1 mg/kg body wt, i.p.) 16 hr before tryptophan injection. Results are means ± S.E. of four observations. Tryptophan dosage: (□) control (saline vehicle); (●) 190 mg/kg; (■) 375 mg/kg; (○) 750 mg/kg).

Table 3. Effect of tryptophan, triamcinolone and endotoxin on total hepatic tryptophan dioxygenase activity in 48 hr starved rats*

	Treatment			Enzyme activity (μmoles/min/g wet liver)	P
	Tryptophan	Triamcinolone	Endotoxin		
I	−	−	−	50 ± 3 (3)	—
II	+	−	−	150 ± 4 (3)	vs I, < 0.001
III	−	+	−	230 ± 16 (3)	vs I, < 0.001
IV	−	−	+	90 ± 8 (6)	vs I, < 0.05
V	+	+	−	510 ± 46 (3)	vs II, < 0.001; vs III, < 0.01
VI	+	−	+	310 ± 39 (5)	vs II, < 0.001; vs IV, < 0.01
VII	−	+	+	110 ± 21 (6)	vs III, < 0.01; vs IV, N.S.
VIII	+	+	+	490 ± 21 (5)	vs V, N.S.; vs VI, < 0.01; vs VII, < 0.001

* Endotoxin was given (1 mg/kg body wt, i.p.) 16 hr, and triamcinolone (5 mg/kg body wt, i.p.) 3 hr before tryptophan (750 mg/kg body wt, i.p.). Animals were killed 3 hr after tryptophan administration; where indicated equivalent vols. of 0.9% (w/v) NaCl were given to controls. The number of independent observations is given in parentheses; N.S., not significant.

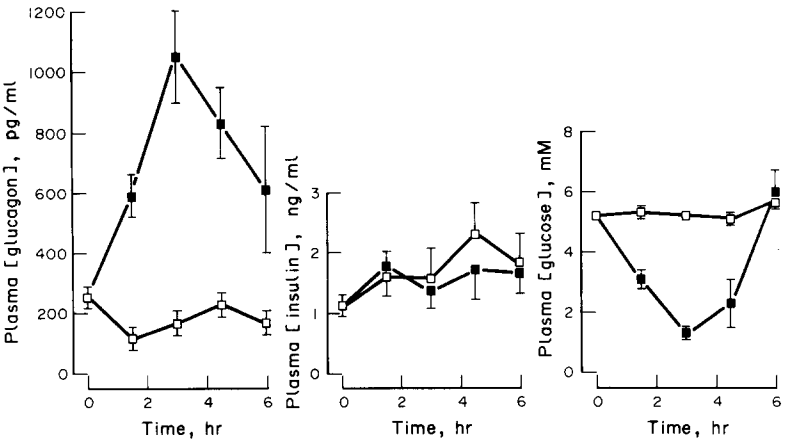

Fig. 4. Effect of a tryptophan load (375 mg/kg body wt i.p.) on plasma glucagon, insulin and glucose concns in 48 hr starved rats treated with endotoxin (1 mg/kg body wt, i.p.) 16 hr before tryptophan injection. Open symbols, controls; closed symbols, tryptophan-treated. Results are means ± S. E. from eight observations. P (vs controls): *<0.05; **<0.01; ***<0.001.

(results not shown). Secondly, measurements of plasma tryptophan concns showed rates of disappearance similar to those seen in control animals (Fig. 3; [45]). Thirdly, the rate of metabolism of L-[ring 2-^{14}C]tryptophan by isolated liver cells from 48 hr starved endotoxin-treated rats was again similar to that by control cells (results not shown).

Although endotoxin may acutely increase plasma insulin concns [37], this effect is no longer seen after 3 hr. Plasma concns of insulin and glucagon in our endotoxin-treated animals 16 hr after injection were similar to those of sham-injected controls (Fig. 4; see ref. [25]). Administration of tryptophan led to a large rise in glucagon, without any change in insulin concns, accompanied by the expected hypoglycaemia. Animals, of this population but not treated with endotoxin previously, did not become hypoglycaemic when given tryptophan but did show the same pronounced hyperglucagonaemia [25]. These results are consistent with a hypothesis that exposure to endotoxin may impair the capacity of the liver to respond normally to glucagon. This would not seen unreasonable in the light of the well-documented permissive role of glucocorticoids for glucagon action and the inhibition of glucocorticoid action itself by endotoxin.

Moon [29] reported that cyproheptadine, a 5-hydroxytryptamine antagonist, blocked the hypoglycaemic action of tryptophan in endotoxicosis and suggested that 5-hydroxytryptamine might be an important effector. Although 5-hydroxytryptamine is known to interact with carbohydrate metabolism in several ways [24, 46, 47], we have been unable to obtain evidence confirming its role in the experiments described above. Pretreatment of animals with *p*-chlorophenylalanine, a chronic inhibitor of tryptophan 5-mono-oxygenase [48], methysergide, a general 5-hydroxytryptamine antagonist [49], or xylamidine, a peripheral 5-hydroxytryptamine antagonist [50], had no significant effect on the hypoglycaemic response. An exception was that some protection was given by methysergide at 5 mg/kg body wt. High doses can, however, produce non-specific effects including those of 5-hydroxytryptamine itself [51]; similar considerations may apply to cyproheptadine, as noted in ref. [29]. MK-486 (carbidopa), an inhibitor of aromatic amino acid decarboxylase [52], was effective in alleviating tryptophan-induced hypoglycaemia, but is known to inhibit other pyridoxal phosphate-linked enzymes, including kynureninase in the tryptophan oxidative pathway [53, 54]. Collectively, these results with inhibitors of tryptophan metabolism suggest that a monoamine is unlikely to mediate the hypoglycaemic response to tryptophan administration, (in endotoxin-treated animals at least).

Inhibition of hepatic gluconeogenesis in rats by tryptophan has been ascribed to the formation of quinolinate from the amino acid through the oxidative pathway [55–57]. Glucose synthesis in liver cells from 48 hr starved endotoxin-treated rats is inhibited to the same extent, and with the same responsiveness, to tryptophan as in control cells (Fig. 5; [25]). Together with the direct measurements of tryptophan oxidation (see above), this suggests that hypoglycaemia in endotoxin-treated rats is not qual-

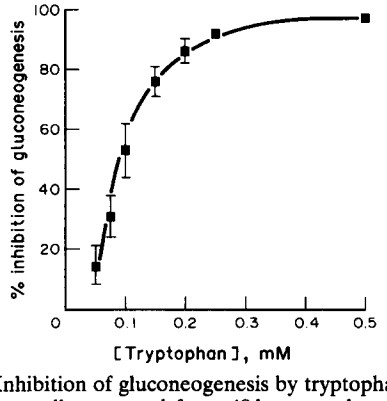

Fig. 5. Inhibition of gluconeogenesis by tryptophan in isolated liver cells prepared from 48 hr starved rats treated with endotoxin (1 mg/kg body wt, i.p.) 16 hr before cell preparation. Substrate, 10 mM L-lactate; rate, 171 ± 6 nmoles glucose/mg dry wt/hr. Results are means ± S.E. from three independent observations.

itatively distinct from that in other predisposed groups of animals.

The mechanism of action of endotoxin at the molecular level remains a matter for speculation. It is possible that there are intermediate steps involving the reticuloendothelial system [20–23] and the production of one or more specific factors [21, 23, 58–60] which, among other actions, oppose glucocorticoid action [21, 23] and may involve α-adrenergic receptors [61]. It seems unlikely, however, that changes in the activity of tryptophan dioxygenase with endotoxin are of primary significance in the development of the subsequent symptoms.

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