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The Protein Synthesis Inhibitor, Anisomycin, Causes Exacerbation of the Iminodipropionitrile-Induced Spasmodic Dyskinetic Syndrome in Rats

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STENNETT, R., M. KATZ, V. JACKSON-LEWIS, S. FAHN AND J. L. CADET. *The protein synthesis inhibitor, anisomycin, causes exacerbation of the iminodipropionitrile-induced spasmodic dyskinetic syndrome in rats.* PHARMACOL BIOCHEM BEHAV **32**(4) 1003–1008, 1989. —The effects of anisomycin on dyskinetic head movements, circling, and locomotor activity were investigated in the IDPN-induced syndrome. Intracerebroventricular (ICV) injections of anisomycin in conjunction with IDPN caused exacerbation of all aspects of the syndrome, although circling and vertical head dyskinesias (retrocollis) were the most affected. Animals treated with only anisomycin showed persistent retrocollis but not laterocollis or circling. Biochemical studies confirmed the increases in the concentration of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) previously observed in the striata of IDPN-treated rats two weeks after stopping administration of the drug. Rats treated with anisomycin alone also showed significant increases in striatal 5-HT and 5-HIAA concentrations which were somewhat higher on the side of the ICV infusions. Coadministration of IDPN and anisomycin did not cause any further increases in 5-HT or 5-HIAA. These results suggest that inhibition of protein synthesis by IDPN may be one of the processes involved in the development of the persistent dyskinetic syndrome.

Iminodipropionitrile Spasmodic dyskinesias Locomotor activity Anisomycin Protein synthesis inhibition Serotonin Striatum

CHRONIC injections of iminodipropionitrile (IDPN) lead to the development of a complex motor syndrome which is characterized by persistent spasmodic dyskinetic head movements, circling, increased startle responses, and other less frequently observed signs in rodents [(15, 20, 21, 30, 31), see (4) for review]. Certain aspects of the syndrome may be more prominent in some species. For example, mice exhibit more persistent rotational behavior and hyperactivity, whereas rats sometimes show mainly the head dyskinesias and increased acoustic startle responses depending on the dose of IDPN used.

It has been suggested that interactions between the norepinephrine (NE), the serotonin (5-HT), and the opiate systems might be important in the development of these abnormalities (4). For example, the use of 5-HT-2 (5), alpha-1 (8), and opiate (7) receptor antagonists caused attenuation of the IDPN-induced dyskinesias in mice. Furthermore, chronic administration of the drug causes increases in the levels of 5-HT in the striatum and the nucleus accumbens (10) and reciprocal decreases in the number of [^{125}I]LSD-labelled 5-HT-2 receptors in the same areas of rat brain (11). There were also changes in the levels of alpha-1 noradrenergic receptors (2) as well as abnormalities in the levels of opiates (9) and their receptors (12) in the rat.

Because the IDPN-induced abnormalities develop after a hiatus of seven days during which the animals received daily injections (4), and because the drug causes changes in neurotransmitters such as 5-HT (10) and the opioid peptides (9) which are linked to various messenger systems (26,27), it was hypothesized that the development and persistence of the syndrome may be related to posttranslational modification of existing proteins (4,13). These ideas were supported by the observation of significant increases in striatal protein phosphorylation in IDPN-treated rats (6). Nevertheless, the possible involvement of altered protein synthesis needed to be considered. The present experiments were carried out in order to evaluate the effects of protein synthesis inhibition on

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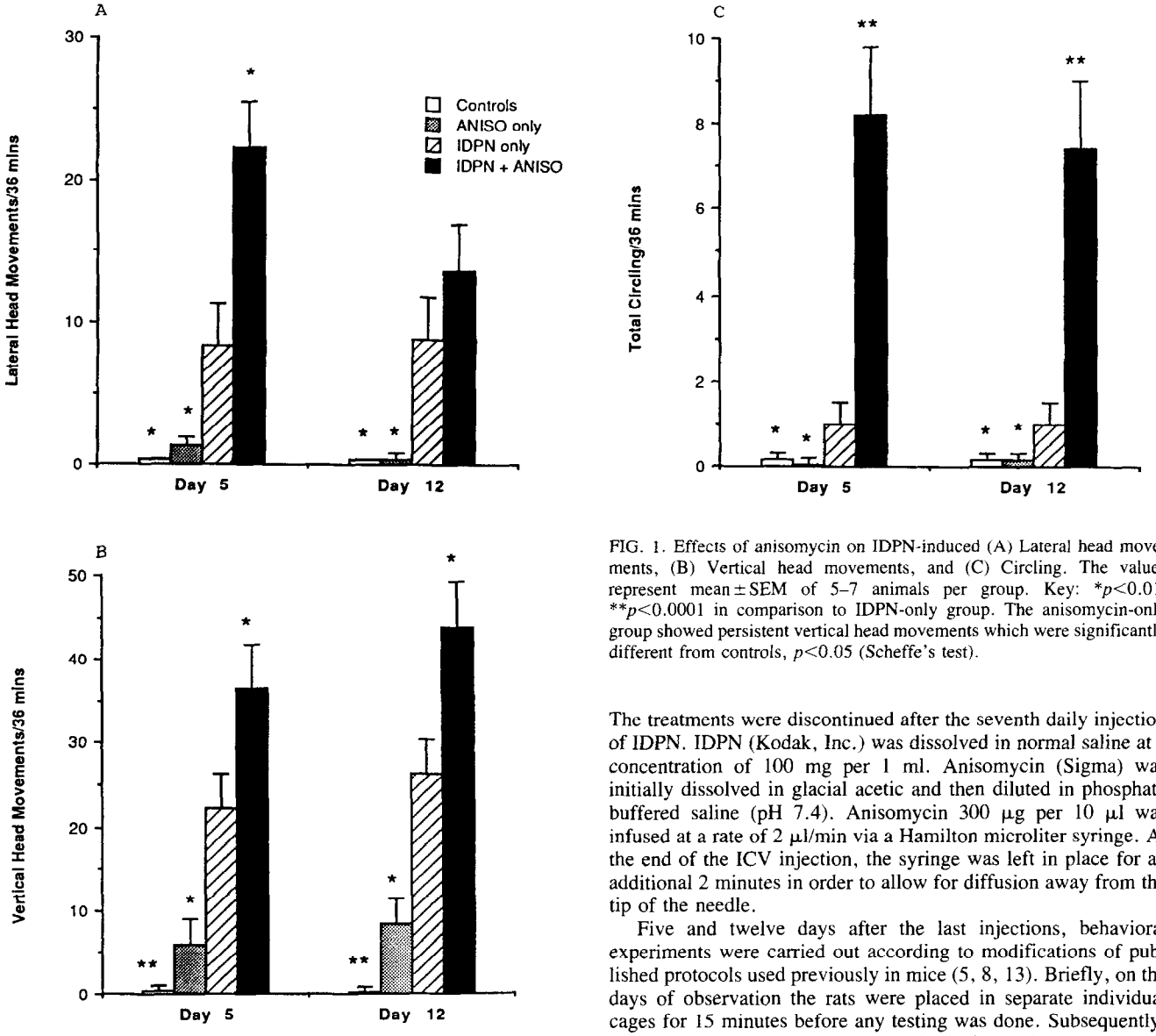

FIG. 1. Effects of anisomycin on IDPN-induced (A) Lateral head movements, (B) Vertical head movements, and (C) Circling. The values represent mean ± SEM of 5–7 animals per group. Key: $*p<0.01$; $**p<0.0001$ in comparison to IDPN-only group. The anisomycin-only group showed persistent vertical head movements which were significantly different from controls, $p<0.05$ (Scheffe's test).

various aspects of the 'waltzing' syndrome (15).

METHOD

Male Sprague-Dawley rats (220–250 g) were used. The animals were kept in a temperature-controlled room with a 12 hour light-dark cycle and were given free access to food and water.

At the beginning of the experiment, each rat was stereotaxically implanted with a permanent stainless steel guide cannula (Plastic Products) into the right lateral ventricle under general anesthesia with chloral hydrate (400 mg/kg). The coordinates were 0.8 mm posterior to bregma, 1.5 mm lateral to the midline, and 3.6 mm ventral to the surface of the animal skull, according to the atlas of Paxinos and Watson (28). The placement was tested by slowly drawing cerebrospinal fluid. After a week of recovery from surgery, the rats were divided into four groups: 1) controls; 2) IDPN (100 mg/kg, IP) + saline (ICV); 3) anisomycin (300 μg, ICV); and 4) IDPN (100 mg/kg, IP) + anisomycin (300 μg, ICV). The anisomycin groups received the protein synthesis inhibitor one hour before and three hours after the daily IP injections of IDPN. The treatments were discontinued after the seventh daily injection of IDPN. IDPN (Kodak, Inc.) was dissolved in normal saline at a concentration of 100 mg per 1 ml. Anisomycin (Sigma) was initially dissolved in glacial acetic and then diluted in phosphate buffered saline (pH 7.4). Anisomycin 300 μg per 10 μl was infused at a rate of 2 μl/min via a Hamilton microliter syringe. At the end of the ICV injection, the syringe was left in place for an additional 2 minutes in order to allow for diffusion away from the tip of the needle.

Five and twelve days after the last injections, behavioral experiments were carried out according to modifications of published protocols used previously in mice (5, 8, 13). Briefly, on the days of observation the rats were placed in separate individual cages for 15 minutes before any testing was done. Subsequently, activity counts were monitored at 6-min intervals as previously described (8,13). Lateral and vertical head movements were counted six times at 6-min intervals over a period of 36 minutes. Circling was rated at the same times on a 4-point severity scale as described (5). For statistical analysis, the mean score for each 6-min interval was added so that the possible total mean severity score for circling was 24.

Forty-eight hours after the last testing period, the animals were sacrificed and the striata were immediately dissected out on ice. The tissues were kept frozen at −80°C until assayed for 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) by high-performance liquid chromatography (HPLC) with electrochemical detection with minor modifications of previously published methods (29,35). Briefly, the samples were weighed and homogenized in 15 volumes of ice cold 0.1 M perchoric acid containing 0.4 mM sodium bisulfite and 0.1 mM EDTA. Dihydroxybenzylamine was used as an internal standard. After a 5-min centrifugation at 4°C, an aliquot (40 μl) of the supernatant was filtered through a 0.45 μM filter and injected into the chromatograph. The mobile phase consisted of 0.15 M monochloroacetic acid, 0.12 M sodium hydroxide, 9% methanol, 0.1 mM EDTA, 0.1 mM sodium octyl

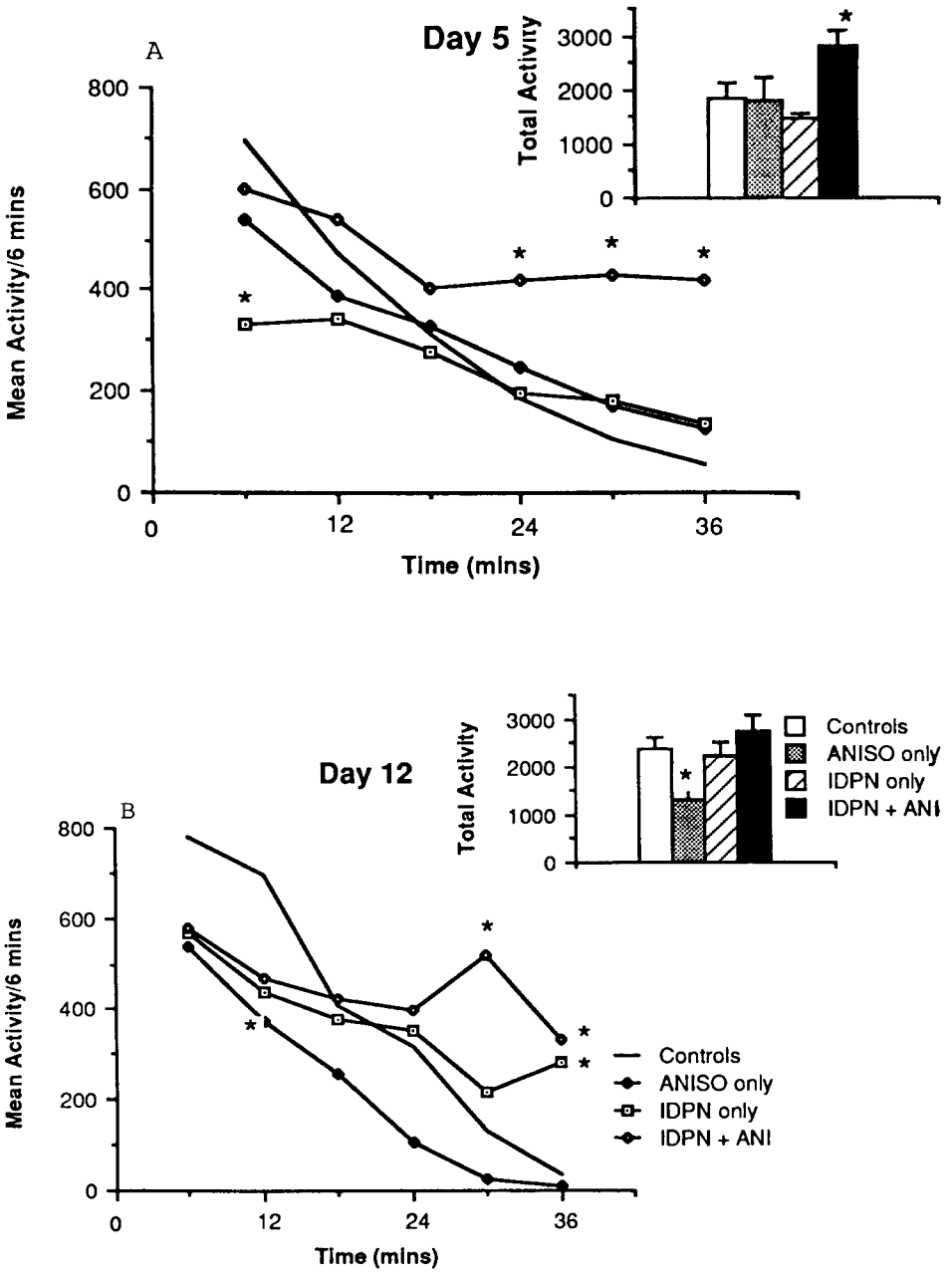

FIG. 2. Effects of anisomycin and IDPN on locomotor activity on (A) day 5 and (B) day 12 after stopping drug administration. The insets represent the total activity obtained during the 36 minutes of observation. Key: *<0.01 in comparison to controls (Scheffe's test).

sulfate, and 1% tetrahydrofuran, pH 3.0. The solvent was run at 0.8 ml/min at room temperature with the potential set at +0.77 V. The sensitivity was set at 2–10 nA/V. The typical retention times for 5-HT and 5-HIAA were 14.1 min and 16.95 min, respectively. The two striata were kept separate in order to test whether anisomycin might have preferential effects on the side where it was infused.

The raw biochemical and behavior data were analyzed by analysis of variance followed by Scheffe's multiple group comparisons. Side differences in the concentrations of 5-HT and 5-HIAA were analyzed by student's *t*-test. The null hypothesis was rejected at the 0.05 level.

RESULTS

Head Movements and Circling

Chronic administration of IDPN caused the development of the spasmodic abnormalities as expected. The coadministration of anisomycin and IDPN caused increases in the manifestation of all the aspects of the syndrome measured in the present study (Fig. 1). For example, lateral head movements were more prominent in the

TABLE 1

EFFECTS OF IDPN AND ANISOMYCIN ON STRIATAL CONCENTRATION OF 5-HT AND 5-HIAA

	5-HT[1]		5-HIAA[1]	
	Ipsi	Contra	Ipsi	Contra
Controls (5)	0.82 ± 0.20	0.86 ± 0.01	0.61 ± 0.02	0.62 ± 0.01
Aniso Only (6)	4.33 ± 0.33[a]	3.23 ± 0.20	2.79 ± 0.17[a]	2.21 ± 0.11
IDPN Only (7)	2.95 ± 0.15‡	2.70 ± 0.15	1.68 ± 0.11†	1.66 ± 0.10*
IDPN + Aniso (5)	3.38 ± 0.21[a,]*	2.96 ± 0.19	2.37 ± 0.19[b]	1.89 ± 0.17

The values represent mean ± SEM (ng/mg tissue) of the number of animals in parentheses. The animals were sacrificed 13 days after the last injection of IDPN and anisomycin. Ipsilateral (Ipsi) and contralateral (Contra) refer to the side of intraventricular injections of anisomycin.

[a]$p<0.05$; [b]$p<0.01$ in comparison to contralateral side (Student's *t*-test).

*$p<0.05$; †$p<0.01$; ‡$p<0.001$ in comparison to anisomycin only group (Scheffe's test).

[1]All the treatment groups had higher concentrations of both 5-HT and 5-HIAA on both sides when compared to the control group, $p<0.0001$ (Scheffe's test).

cotreatment group during the first but not during the second testing session (Fig. 1A), whereas vertical head dyskinesias were more marked during both times (Fig. 1B). In addition, infusion of only anisomycin caused the appearance of vertical head dyskinesias which were not as prominent as those caused by IDPN alone (Fig. 1B). Interestingly, the effects of coadministering anisomycin with IDPN on IDPN-induced circling were even more remarkable since IDPN alone resulted in minimal rotational behavior (Fig. 1C).

Activity

The normal animals showed a somewhat higher level of activity than the other groups when put in a novel environment (Fig. 2). The total activity counts of the IDPN-alone or of the anisomycin-alone groups were not significantly different from normal controls during the first testing period (Fig. 2A, inset). These three groups showed a time-dependent reduction in locomotor activity during the 36 minutes of observation. In contrast, the anisomycin + IDPN group showed a failure to habituate during the same time span. In addition, the total activity counts for that group were higher than those of the other groups during the first testing session (Fig. 2A, inset). The results for the second testing period were somewhat different from those of the first (Fig. 2B). For example, anisomycin caused a marked reduction of activity in comparison to the other groups on the second testing session, a result that is similar to those of Squire and Barondes (36). In addition, although both the IDPN-only and the IDPN + anisomycin groups showed a relative failure to habituate to a novel environment, the total activity counts for these two groups were not different from that of the normal control group due to the observation that the control group had higher activity counts at the beginning of the testing period.

Biochemical Studies

As reported previously (10), IDPN causes significant increases in the levels of striatal 5-HT and 5-HIAA in IDPN-treated rats (Table 1). The coadministration of IDPN and anisomycin caused significant increases in levels of 5-HT and 5-HIAA which were somewhat higher on the side of injection of anisomycin. Anisomycin alone also caused increases in both 5-HT and 5-HIAA in the striatum. These changes were also higher on the injected side. Moreover, the increases in 5-HT observed on the ipsilateral side were significantly higher than those observed in either the IDPN only or the IDPN + anisomycin group. The increases in 5-HIAA caused by anisomycin were significantly higher than those induced by IDPN alone on both the ipsilateral and contralateral sides.

DISCUSSION

The protein synthesis inhibitor anisomycin caused significant increases in IDPN-induced circling, and lateral and vertical spasmodic head dyskinesias. Moreover, the coadministration of these two compounds caused a persistent elevation of activity counts five days after the last injection of the drugs. The failure to habituate observed in the IDPN + anisomycin group is similar to what has been observed in IDPN-treated mice (8,13). The present observations implicate a role for inhibition of protein synthesis in the development of some aspects of the IDPN-induced persistent dyskinetic syndrome. Protein synthesis inhibitors have previously been shown to effect, to various degree, spontaneous activity in rodents (36,37). Thus, the exacerbation of the IDPN-induced syndrome may be related to a potentiating effect of anisomycin on the molecular events which are associated with the development of the persistent circling and spasmodic abnormalities.

Our results are in conflict with those of a previous study in which the protein synthesis inhibitor ethionine was reported to prevent the development of the IDPN-induced abnormalities in rats (3). There are, however, a number of differences between that study and ours. First, one large dose (2 g/kg) was used by the other authors (3), whereas we used 7 daily injections of 100 mg/kg of IDPN. Second, they used the 'swim' test which consisted only of observing the swimming patterns of rats injected with IDPN alone or with ethionine plus IDPN (3), whereas we looked at specific aspects of the syndrome. Because of the discrepancies between their results and ours, we tried, nevertheless, to replicate their findings using the daily injections of a smaller dose of IDPN which have been proven in our hands to cause less systemic toxicity. This attempt was unsuccessful because the use of similar or lower doses of ethionine used by the other authors was associated with significant acute toxicity and death. In any case, since the mechanisms of action of anisomycin (23) and ethionine (17,34) are different, it is still possible that the preventive effects reported by the other group may be related to other properties of ethionine. For example, anisomycin blocks the formation of peptide bond by eucaryotic cytoplasmic ribosomes (23), whereas ethionine interferes with the incorporation of methionine and glycine into proteins (34), inhibits the conversion of methionine to cystine (34), and causes increases in the lipid content of the liver (17).

The consistency of the data for all aspects of the syndrome examined suggests a role for molecular processes related to protein synthesis in the development of these persistent signs. The lack of habituation observed after coadministration of anisomycin and IDPN also implicates possible inhibition of the synthesis of proteins which might be related to learning (36). Since IDPN has been shown to affect both anterograde (22) and retrograde (18) axonal transport, further inhibition of the synthesis of these proteins by anisomycin might have potentiated the deleterious effects of IDPN on neurochemical and neuroanatomical pathways responsible for the proper functioning of motor systems. The possibility that anisomycin might have exacerbated the effects of IDPN on axonal transport needs also to be taken into consideration since it has previously been shown that anisomycin can interfere with the transport of glycoprotein components from the cell body

into the axon (1). In any case, the present results and previous data on protein phosphorylation (6) raise the possibility that both translational and posttranslational abnormalities may play a role in the development of the IDPN-induced persistent spasmodic syndrome. The possibility that IDPN alone or in combination with anisomycin might have affected DNA repair mechanisms needs also to be considered since Shinozaki and Ishida recently reported that a single injection of the anti-DNA antibiotic (38), pepleomycin, causes an irreversible motor syndrome which is almost identical to the IDPN-induced abnormalities in mice (33).

The results of the biochemical studies are in agreement with our previous findings that IDPN injections cause a persistent increase in the levels of striatal 5-HT and 5-HIAA (10). If the dyskinesias were only related to changes in the serotonergic system, then coadministration of IDPN and anisomycin should have caused a greater increase in the level of 5-HT. Since this was not the case, the data suggest that IDPN and anisomycin might be affecting other systems such as the nigrotectal, pontine, or reticulospinal pathways which are involved in circling and head orienting behavior (14, 16, 24).

The increase in striatal 5-HT and 5-HIAA in animals treated with anisomycin alone was unexpected, but may be related to the increased number of vertical head movements that these rats showed. Previous studies on the biochemical effects of anisomycin had reported that the drug causes decreases in the activity in a number of enzymes that regulate the synthesis of neurotransmitters including tyrosine hydroxylase, tryptophan hydroxylase and acetylcholinesterase (19, 32, 39). This inhibition is associated with increases in the levels of the amino acid precursors such as tyrosine and tryptophan (32). Since the initial inhibition of protein synthesis has been shown to be associated with subsequent rebound (25), a similar phenomenon might have led to increase synthesis of 5-HT due to the accumulation of the precursor tryptophan since the biochemical studies were done several days after stopping the injection of anisomycin.

Finally, the present data provide evidence that protein synthesis inhibition with anisomycin causes exacerbation of the IDPN-induced syndrome. These data strengthen the hypothesis that IDPN may cause molecular events in the central nervous system which are responsible for the persistent nature of dyskinetic changes observed after the drug. Elucidation of the specific identity of these changes will await further studies.

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