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The effects of melatonin injection on glucose tolerance in intact and pinealectomized laboratory rats

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THE EFFECTS OF MELATONIN INJECTION ON GLUCOSE TOLERANCE IN INTACT AND PINEALECTOMIZED LABORATORY RATS

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INTRODUCTION

The pineal is a small gland (1.0 mg. in rats) located between the cerebral hemispheres. It contains a variety of active indoleamines and monoamines, the major one being melatonin, a seratonin derivative. The concentration of biosynthetic enzymes within the pineal, and the secretion of melatonin from the pineal exhibits a circadian rhythm as seen in Fig. #1 (Brooks, et. al., 1975).

Melatonin is synthesized from the amino acid tryptophan through a series of enzyme and co-factor specific reactions (Fig. #2). Tryptophan is first hydroxylated at the five position through the action of tryptophan hydroxylase. 5-OH tryptophan is rapidly decarboxylated by dihydroxyphenylalanine decarboxylase to 5-OH tryptamine (serotonin). This compound is an important neurotransmitter found in large concentrations in specific areas of the central nervous system, including the pineal. N-acetylase converts serotonin to N-acetyl serotonin. N-acetyl serotonin has a much greater affinity for the enzyme hydroxy indole o-methyl transferase (HIOMT) than does serotonin. HIOMT is found almost exclusively in the cytoplasm of the pineal parenchymal cells, and transfers an active methyl group from s-adenosyl methionine to the 5-position of this melatonin presursor. One major physiological effect of the action of HIOMT is to convert indoleamines such as serotonin

which cannot cross the blood barrier, into compounds such as melatonin which easily gain access to the brain.

As shown in Fig. #2 each enzymatic step for the formation of melatonin from typtophan requires a different specific co-factor. Tryptophan hydroxylase requires NADPH and tetrahydrobiopterin. Pyridexyl phosphate (vitamin B_6) is necessary as a co-factor for the action of dihydroxy-phenylalanine decarboxylase. Acetyl groups for the N-acetyl-ation of serotonin come from acetyl CoA, while S-adenosyl methionine donates the methyl group for the O-methylation of N-acetyl serotonin. Formation of melatonin from tryp-tophan in pineal organ cultures, without the addition of co-factors, indicates that this organ contains, or is capable of synthesizing, sufficient amounts of these com-pounds for the production of melatonin (Axelrod, 1974).

Light has a major regulatory effect on pineal indoleamine biosynthesis and secretion. A circadian rhythm (Fig. #1) is exhibited by the pineal secretions. Connection between the eye and the pineal follows a direct sympathetic pathway (Axelrod, 1974). Maintenance of melatonin biosyn-thetic activity requires continuous occupation of the adrenergic receptors by the sympathetic neurotransmitter norepinephrine. Light reduces the release of norepinephrine from the sympathetic fibers and a rapid fall in melatonin production and secretion ensues. As can be seen from Fig. #1

the concentration of melatonin secreted by the pineal reaches its lowest level about three hours after the onset of daylight. One hour after darkness there is a 30 to 50 fold increase in pineal N-acetylast activity (Binkley, 1976). This increase in pineal N-acetylase activity after the onset of darkness is responsible for a 10 times greater concentration of melatonin at night above daytime values (Romero, et. al., 1975b). Melatonin concentration reaches a peak approximately three hours after the onset of darkness (Axelrod, 1974).

β adrenergic blocking agents inhibit indoleamine biosynthesis and secretion, while α adrenergic blocking agents effect no change (Klein & Weller, 1973). β adrenergic stimulation results in the activition of adenyl cyclase to produce 3'4' cyclic adenosine menophosphate. This suggests that melatonin biosynthesis and secretion are another of the more than 30 hormonal reactions known to be mediated by cyclic AMP (Romero, et. al., 1975a).

A number of different experimental techniques have shown a relationship between melatonin, glucose tolerance, and insulin release. In vivo studies have indicated an inhibition of insulin release by many monoamines such as epinephrine (Kriss, 1966), and norepinephrine (Porte & Williams, 1966). L-dopa and dopamine also increase blood glucose levels, suggesting a reduction in the release of

insulin (Lundquist, 1972). Serotonin has also been shown to inhibit glucose mediated insulin release (Feldman & Lebowitz, 1972; Feldman, et. al., 1972). Melatonin has been implicated in decreasing the basal insulin secretion rate from cultured rat islet cells (Bailey, 1974; Csaba & Barath, 1971; Milcu, et. al., 1971; Csaba & Nagy, 1971). In vivo studies on anesthetized cannulated rats have shown a slight decrease in glucose tolerance due to intravenous melatonin administration (Bailey, 1974).

A monoaminergic mechanism has been proposed to explain this inhibition of insulin release (Lundquist, 1972), but the exact details of the mechanism are not presently understood. Treatment with monoamine oxidase (MAO), which degrades monoamines, decreases the active concentration of monoamines. Treatment with MAO inhibitors produces a decrease in the degratory activity of the MAO system, and therefore allows monoamines to effectively increase in concentration. This increase in monoamine activity produces a decrease in insulin production and/or release, thereby producing an increase, or perhaps more accurately, a lack of decrease in plasma glucose (Lundquist, 1972).

Studies on rat islet preparations have shown melatonin inhibits MAO activity and thereby reduces glucose mediated insulin release (Ellis, 1976). The objective of this work is to investigate the effects of melatonin on

insulin release, and on glucose mediated insulin release in the intact and in the pinealectomized rat.

METHODS AND MATERIALS

A total of 75 rats were obtained from random bred long Evans stock. Twenty-five were used as controls, and pinealectomies were performed on the remaining 50 animals using a modification of two techniques (Hoffman & Reiter, 1965; Bliss & Bates, 1973). The animals were anesthetized with ether and the skin and fascia overlying the skull were opened by longitudinal incision. A 0.5 cm. wide disc was cut in the skull using a dental drill with a thin-walled, tube-shaped bit. This bone disc was removed from the skull with a pair of forceps, leaving an opening in the skull anterior to the lamboidal suture and lateral to the saggital suture. Microforceps, opened 2 to 3 mm., were then inserted into the cranium at an angle of approximately 45^{o} downward and 45^{o} lateral to the saggital suture, rupturing the saggital sinus just anterior to the transverse sinus. The forceps were then moved approximately 3 mm. medioposteriorly, closed around the unexposed pineal, and retracted through the entry path. Although the pineal was not visible through the skull opening with this technique, it has the advantage that bleeding was kept to a minimum. The bone disc was replaced and the skin incision closed using metal sutures. Using this technique the fatality rate was 4%.

The pinealectomized rats were divided into two test groups. The animals in one group (P+M) were injected with

melatonin (Sigma) intraperitoneally at a dose of 500 mg./kg. body wt., at 2200 hours daily for three days prior to any test. The pinealectomized animals making up the other group (P) were not injected with melatonin. A third group of animals upon which no operation had been performed (C) served as controls. Two test treatments were run on each test group: (1) glucose tolerance test; (2) saline control test (Table 1). For the glucose tolerance test a 20% glucose solution was administered intraperitoneally at a dose of 300 mg./kg. body wt. Locke-Ringers solution was injected (1.5 ml./kg. body wt.) for the saline control tests. The effects of pineal secretions within each rat were standardized by starting all tests within one hour of 1000 hours. Blood samples were collected in heparinized capillary tubes using the tail-cut method 30 minutes, 15 minutes, and immediately prior to the injection of the glucose or saline solution. All samples were contrifuged in a microhematocrit (International Equipment Co.) for 5 minutes, the packed red cells discarded, and the plasma tested for glucose concentration. Plasma glucose levels were estimated using the glucose oxidase method (Sigma, 1974). Plasma glucose levels were converted to percent change from each animals lowest pre-injection level. By using this method differences that occurred in initial plasma glucose were standardized.

Some pinealectomized animals were used for more than

one test in the (P) or (P+M) groups. If a second test was performed on an animal, at least two weeks were allowed after the first test was completed. In these cases the animal was used in the (P+M) group last so the injection of melatonin would not interfere with later tests. No animal was used in the same group for the same test more than once.

RESULTS

Following the injection of the glucose solution all animals exhibited a rapid increase in plasma glucose, reaching a peak approximately 15 minutes after the injection (Fig. #3). The magnitude of the increase in the percent change in "mean plasma glucose" (MPG) in the (P+M) group (65%) was greater than the increase in either the (P) group (38%) or the (C) group (33%). The percent change in MPG of all three groups then decreased, stabilizing about 90 minutes post-injection. The percent change in MPG of the (P+M) group stabilized approximately 20% higher than the other two groups. MPG concentrations for the (P) and (C) animals stabilized about 5% below their pre-injection levels, while that of the melatonin injected animals, (P+M), stabilized around 15% above their pre-injection levels.

An increase in MPG followed the injection of saline in all three groups. The increase of the (P+M) group was approximately 30%, while both the (P) and (C) groups MPG increased less than 10%. The MPG values of all three groups then decreased and eventually stabilized about 90 minutes following the saline injection. The MPG of the (P) and (C) groups stabilized about 10-12% below their pre-injection level while that of the (P+M) group leveled off about 15% above their pre-injection level, which is 25% higher than the final value of the other groups.

Although injection of both glucose and saline caused an increase in the MPG of all groups (Fig. #3), the extent of this increase varied considerably between groups during the glucose tolerance and saline control tests. Injection of glucose injection caused a greater increase in MPG in all test groups than did saline injection. After the administration of glucose the MPG of the (P+M) group increased approximately 65% over the lowest pre-injection level. Following the saline injection the MPG of the (P+M) group increased 32%. The (P) group increased its MPG 39% after the glucose injection, and only 8% following the saline injection. The MPG of the (C) group increased 33% following the glucose injection and 5% after the saline injection. Each group shows an increase in MPG of between 30% to 35% higher following the glucose injection when compared to the increase in MPG following the saline injection.

F-tests showed no significant difference between sexes during either the glucose or saline tests, so the sexes were treated together (Table #1). The F-test did show a significant difference within the three groups, (P+M); (P); (C), during the glucose test and during the saline test (Table #1). Group variations within each test were determined by the use of T-tests. As can be seen from Table #2 the (P) and (C) groups showed no significant

variation between each other in either the glucose or saline test. The (P+M) group varied significantly from both the (P) and (C) groups in both the glucose and the saline tests.

Broken capillary tubes or insufficient volumes of plasma samples caused a few missing values. These missing values were estimated by the use of regressions. Correlation co-efficients were run to determine which type of regression more closely represented each groups test data. Linear regressions showed the closest correlation and were used to estimate the missing values for both the glucose and saline treated animals.

Autopsies were performed on all animals to verify removal of the pineal. Data from any animal which still had any portion of the pineal remaining were discarded.

DISCUSSION

The (P+M) group exhibits a reduced glucose tolerance relative to the (P) group. (Fig. #3) The only experimental difference between these two groups was the nocturnal injection of melatonin given the (P+M) group. The difference in change in plasma glucose suggests that melatonin is responsible for the observed glucose intolerance, and therefore, inhibits insulin biosynthesis and/or release. If melatonin did not influence glucose metabolism the percent change in plasma glucose of the two groups would have been similar.

The relationship between the (P+M) group and the (C) group is much more complex. Both groups have melatonin introduced during the night hours. The (C) group has melatonin secreted from the pineal in response to the onset of darkness. The (P+M) group received an injection of melatonin at 2200 hours, approximately when the (C) group experiences its largest secretion rate of melatonin. However, these groups vary in glucose tolerance when tested at 1000 hours. A significantly greater amount of melatonin may be present in the systems of the (P+M) group, due to the injection of melatonin, to effect the noted glucose intolerance at 1000 hours. The (C) group may have metabolized physiologically active levels of melatonin from their blood while the (P+M) group may still have active concentrations in their systems

from the injection.

At the tolerance test starting time the melatonin concentration in the (C) animals is at the low point of their circadian cycle. The (P) group does not have physiologically active levels of melatonin due to the removal of the pineal gland. These two groups exhibited no significant variation in glucose tolerance during the test. In the (C) group physiologically active amounts of the nocturnally secreted melatonin must be metabolized by the liver so that the concentration of melatonin present at 1000 hours is not significantly greater than the melatonin levels of the (P) group. In both the (P) and (C) groups melatonin levels are not high enough to significantly affect glucose metabolism at 1000 hours.

The lack of difference between the (P) group and the (C) group and the significant difference between both and the (P+M) group suggests that while the normal nocturnal secretions of melatonin are metabolized by the liver to below physiologically active concentrations the injected dose of melatonin is not reduced to the same low levels and a reduction in glucose tolerance ensues in the injected rats.

An increase in plasma glucose is observed in all three groups following the injection of the saline solution (Fig. #4). This increase in mean plasma glucose, 32% for the (P+M) group, 8% for the (P) group, and 5% for the (C)

group is not as large as the increase seen in the glucose tolerance test and is most likely due to the stress of the experimental procedure. All plasma glucose curves reach a peak about 15 minutes after the saline injection. As in the glucose tolerance test a large variation occurs in the magnitude of this increase between the melatonin injected group (P+M) and the other two groups, (P) and (C). While the increase in MPG of the (P) and (C) groups are not significantly different, ($T<\{0.01\}$), both vary significantly from the (P+M) group ($T>\{0.01\}$). The normal release of insulin in response to the increase in plasma glucose levels elicited by the stressful situation allowed the MPG of the (C) group to increase only 5%. The increase in MPG of the (P) group, 8%, did not vary significantly from this. The amount of melatonin injected into the (P+M) animals resulted in apparently greater than normal levels of melatonin, as the MPG of the (P+M) group increased 35%. This increase in MPG suggests an inhibition of the islets response capacity, mediated by melatonin. After 90 minutes the MPG of the (P) and (C) groups decreased and stabilized approximately 10% below the lowest pre-injection level, while that of the (P+M) group stabilized at 15% above the lowest pre-injection level. This 25% difference in stabilization levels suggest a suppression of insulin production and/or release in the melatonin injected animals.

The most probably explanation for the difference in MPG of the (P+M) group from both the (P) and (C) groups during the glucose tolerance and saline control tests is alterations in the monoamine system caused by the melatonin injection. Melatonin is degraded by the MAO system. By increasing the concentration of melatonin the effectiveness of the MAO system in degrading other monoamines is reduced. A decrease in the degratory activity of the MAO system would increase the activity of monoamines. The relative increase in monoamine activity could cause the observed decrease in glucose tolerance. This mechanism appears to be the most plausible reason for the inability of the melatonin injected pinealectomized animals to reduce their mean blood glucose concentrations to normal levels during the two hours following the test injection.

SUMMARY

The pineal is an active endocrine gland converting a neural input into a hormonal output. Secretion of monoamines from the pineal is regulated by light and exhibits a circadian rhythm. Melatonin, the major pineal secretion, has been implicated in altering insuline production and/or release, and in reducing glucose tolerance.

Intact animals exhibit a specific glucose tolerance at 1000 hours. The glucose tolerance of pinealectomized animals at the same time is not significantly different. The normal production and secretion of melatonin does not significantly alter glucose metabolism at 1000 hours, but the injection of an apparently supraphysiological dose of melatonin at 2200 hours for each of three days prior to the tolerance test significantly reduced glucose tolerance.

This study has shown a relationship between melatonin injection and reduced glucose tolerance in the laboratory rat. Although the exact mechanism by which melatonin acts on glucose tolerance has not been ascertained, a reduction in insulin production and/or release is the probable cause of the decreased glucose tolerance. A monoaminergic mechanism involving melatonin seems the most likely means by which this action is effected.

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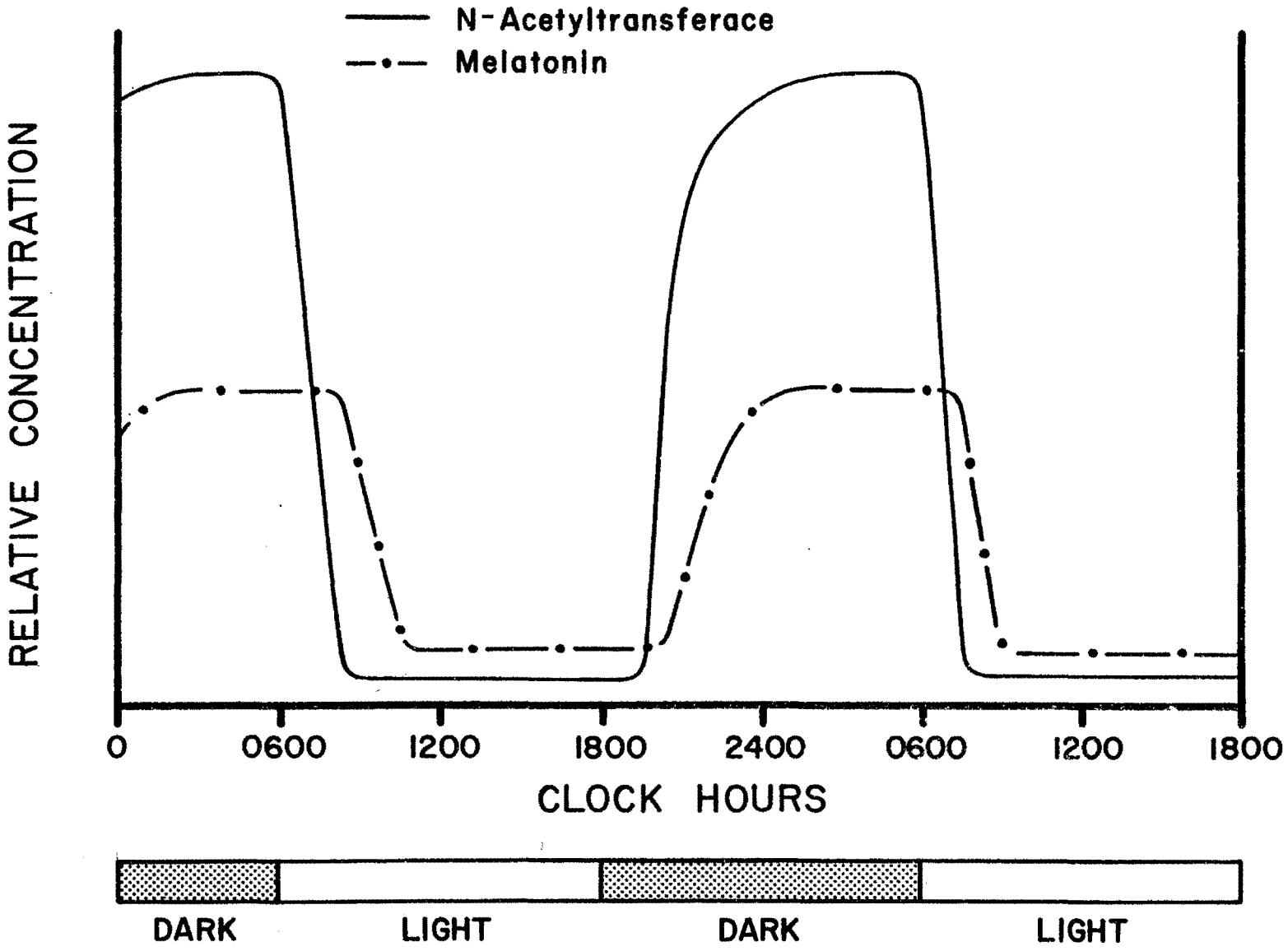
	Source	df	ss	ms	F	
Glucose Tolerance Test	Groups	2	2.293	1.146	8.276	$F_{2,27}=5.5$
	Sexes	1	.107	.107	.774	$F_{1,27}=7.7$
Saline Control Test	Groups	2	2.170	1.085	13.478	$F_{2,27}=5.5$
	Sexes	1	.027	.027	.339	$F_{1,27}=7.7$

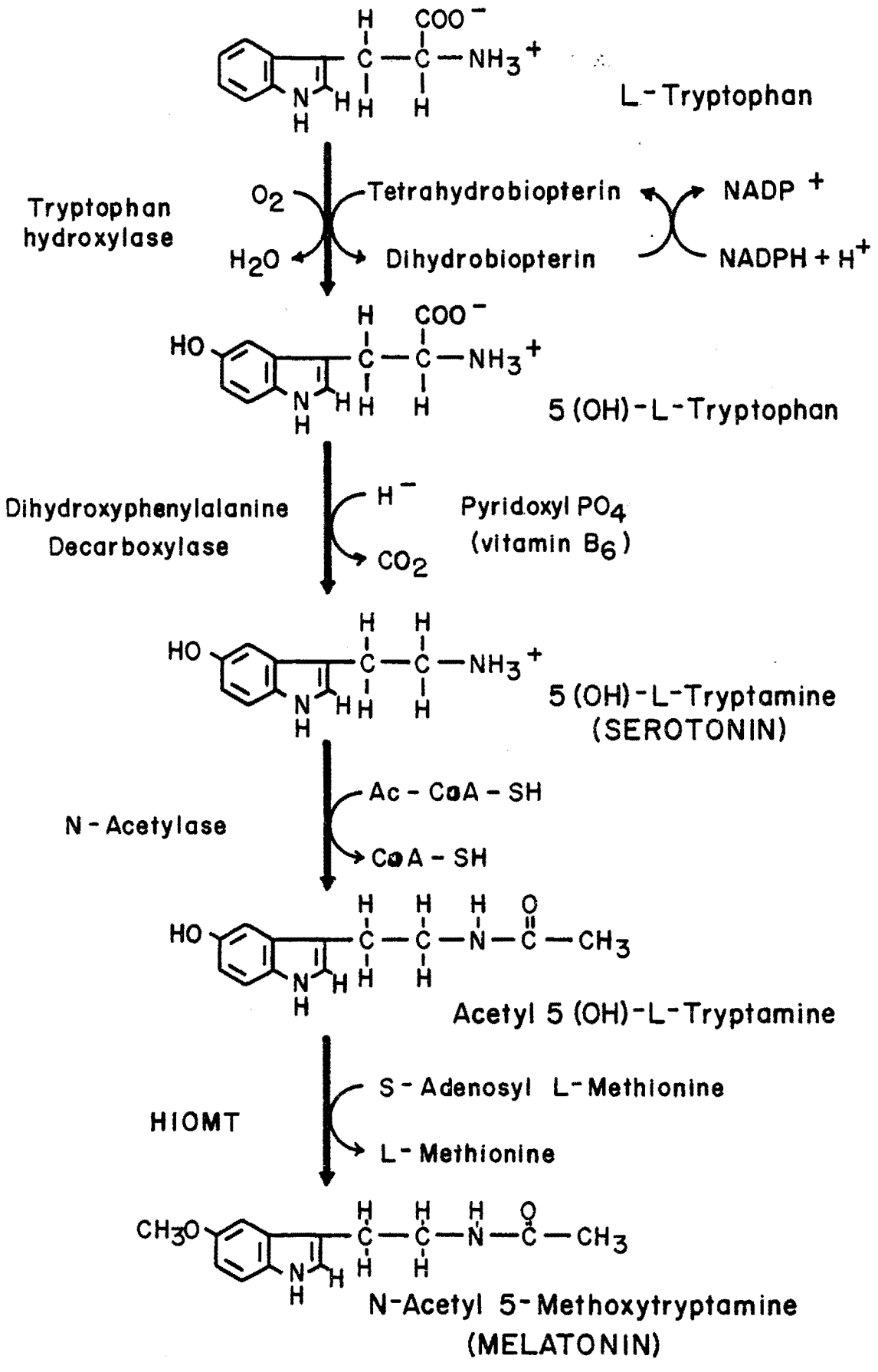
Table 1. Group variations between the three test groups, (P); (C); (P+M), within each test.

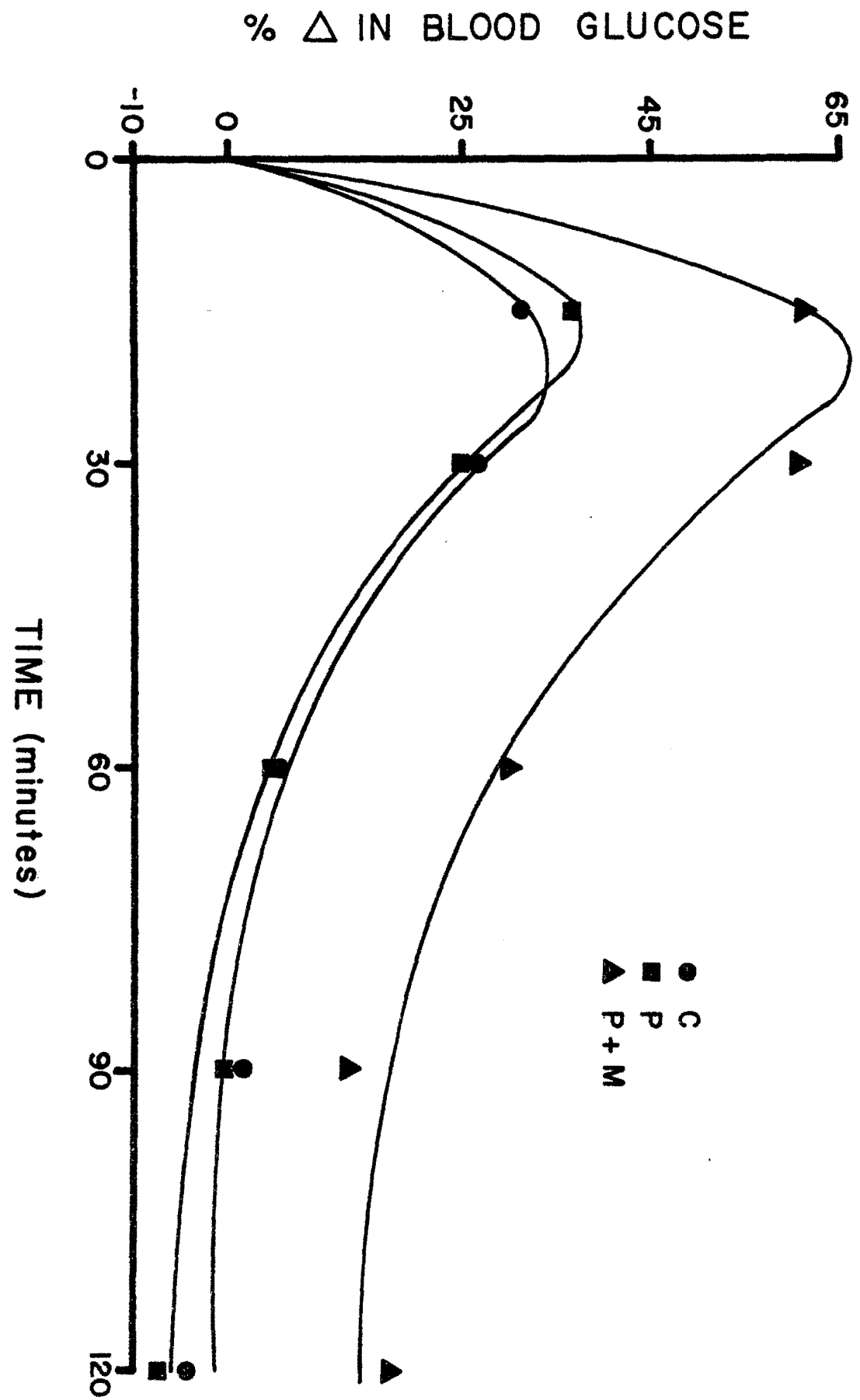
Glucose	DF	T-Value	
C x P	21	0.887	T 0.01 = 2.83
P+MxC	22	3.678	T 0.01 = 2.82
P+MxP	20	6.950	T 0.01 = 2.85
Saline			
C x P	20	1.800	T 0.01 = 2.85
P+MxC	21	14.500	T 0.01 = 2.83
P+MxP	20	31.800	T 0.01 = 2.85

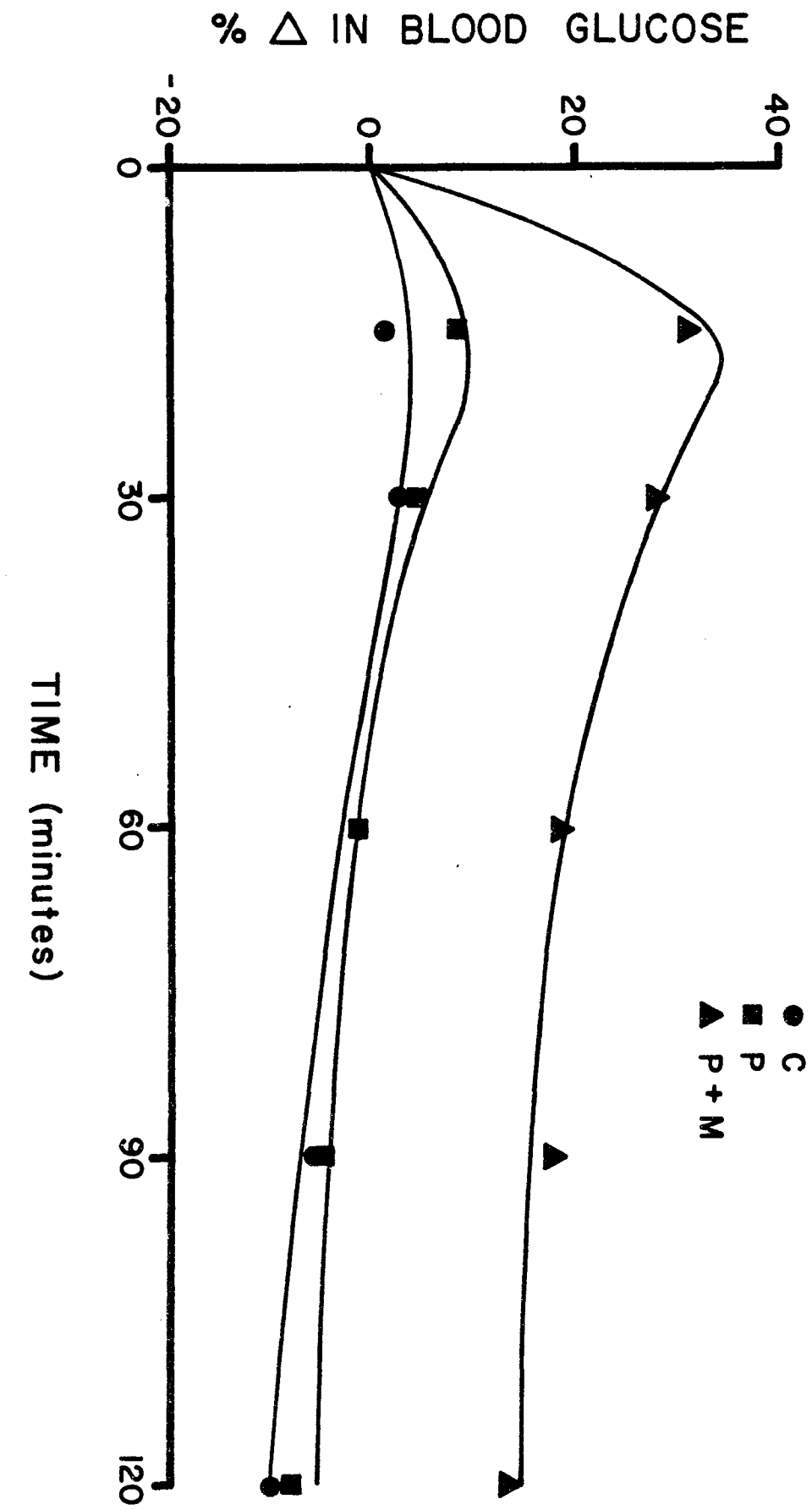
Table 2. Variation between paired test groups within each test using students T-test.


N-Acetyltransferace
Melatonin
RELATIVE CONCENTRATION
0
0600
1200
1800
2400
0600
1200
1800
CLOCK HOURS
DARK
LIGHT
DARK
LIGHT

MELATONIN BIOSYNTHESIS & METABOLISM




% Δ IN BLOOD GLUCOSE
-10
0
25
45
65
0
30
60
90
120
TIME (minutes)
C
P
P+M


% Δ IN BLOOD GLUCOSE
-20
0
20
40
0
30
60
90
120
TIME (minutes)
C
P
P+M