The Effect of Dietary Wheat Protein on Rat Behavior

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Summary

After oral administration of wheat proteins to rats maintained on "normal" cereal containing diet and "wheat-free" rats, the latter had an increased incidence of stereotyped behavior after 10 mg/kg i.p. amphetamine injection: Immunological assay for the wheat protein antigen showed an increased amount in the tissues of "wheat-free" rats although some was also detected in the tissues of "normal" rats.

Introduction

The influence of dietary proteins on brain metabolism and behavior has attracted great interest over recent years, and investigators are now concentrating on the food intake/behavior pattern. Recent evidence has suggested that dietary wheat protein may act as a pathogenic factor in abnormal states in humans (Dohan, 1978; Singh and Kay, 1976).

There are no parallel abnormal behavior states in animals, but the stereotyped behavior produced by high doses of amphetamine is thought to have features in common with some psychotic states (Ran-drup and Munkvaad, 1967; Taylor et al., 1974). This offers a means of experimental investigation of the action of dietary proteins on abnormal behavior in the rat, (Taylor, 1978) in experiments with rats in model psychosis using amphetamine, methionine, tryptophan and wheat gliadins showed that the latter had a marked effect on the behavior pattern.

A recent communication from this laboratory (Hemmings, 1977) has shown that when a number of soluble proteins, including alpha-gliadin labelled with radio-iodine are fed to adult rats, significant amounts of the proteins are to be found in all the organs, including the brain. They are present as large breakdown products (BDP) and to some extent retain antigenicity.

Much work has also been published on the transport of proteins and other antigens from the intestinal lumen of the adult rat, (Walker et al., 1975; Worthington et al., 1974; Williams, 1978; Hemmings and Williams, 1978). These proteins reach the circulation of the animal as antigenic protein and smaller derivatives.

The present work describes experiments on feeding wheat proteins to adult rats, the effect of the protein on amphetamine

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induced behavior, and the subsequent identification of the protein in the tissues throughout the body by serological methods.

Materials and Methods

The subjects were adult male Wistar rats weighing approximately 300 grams, housed individually under normal lighting conditions with water available ad lib. The "normal" rats were maintained on PRD laboratory diet, containing wheat proteins, whilst the "wheat-free" rats were maintained on commercial cat food not containing any wheat or other cereals, carrots and fruit, for periods of six months prior to experiment.

Antiserum to wheat proteins was raised in sheep, by an initial injection of 2 mis of a 1% wheat protein solution mixed with an equal volume of Freund's adjuvant. One month after, and at fortnightly intervals thereafter, further injections of alum precipitated protein were given. After three injections the sheep serum contained antibody to wheat proteins at a titre of 5120 when tested in agar gel.

Experimental groups consisted of six to eight rats. After 24 hours food deprivation the animals were given a 5 ml dose of a 2% wheat protein solution by gavage using a polythene cannula directly into the stomach. Three hours after, each rat was given an injection of 10 mg/Kg D-amphetamine (i.p.) in a volume equivalent to 1 ml/kg body weight. Immediately after amphetamine injection, each rat was individually placed in an evenly illuminated open field (30 cms X 45 cms) where its behavior was observed and recorded for one minute periods every five minutes, until seventy five minutes after the injection.

An all or nothing system of recording was employed with the incidence of the following behavior categories being noted: Active, Rearing, Walking, Sniffing, Immobile, Grooming, Head Movements, Circling, Back Walking, and Side Walking.

On completion of the behavioral experiments, the rats were killed by chloroform inhalation. The blood serum was collected, and the liver, washed intestine, brain and carcass were individually macerated in phosphate buffered saline at a volume of 1 ml/gram weight of tissue using an MSE top drive homogenizer. The homogenates were clarified by spinning at 5000 rpm in an MSE Highspeed 18 centrifuge. The supernatent and serum were subjected to diffusion in agar gel against the specific antiserum using the method of Ouchterlony (1967). Diffusion was allowed to proceed for 72 hours in covered petri dishes when the final titre was recorded.

Results

All the conditions produced a significant increase in the active behavior of "normal" and "wheat-free" animals after administration of wheat protein, showing enhanced activity to that of the usual amphetamine stereotyped behavior pattern. The latency of onset was shorter after administration of wheat protein to "wheat-free" rats than in the "normal" rats, with both types showing more activity than control rats that had only received an amphetamine injection. These behavioral patterns are set out in Table 1.

From the table, the active behavior for the control rats after administration of amphetamine started 25 minutes after the injection and showed increased behavior after 40 minutes, this being typical of amphetamine induced behavior.

Control rats taken from the "wheat-free" stock behaved very similarly, and are not included in the table. This suggests that the variation in diet did not have any adverse effect on the rats' behavior.

The behavior of the "normal" rats after feeding wheat protein was different from the usual stereotyped pattern with onset of behavior starting sooner, at 15 minutes, with much more activity recorded throughout, especially the head movements, circling, back and side walking.

With the rats maintained on the "wheat-free' diet the picture was quite different showing onset of behavior after 15 minutes; after 25-30 minutes, the animals showed much more activity, in particularly the circling, back and side walking categories.

TABLE 1
BEHAVIOR OF AMPHETAMINE TREATED RATS

TREATMENT		BEHAVIOR										
DIET TEST DOSE	NO. TESTED	TIME (mins)	ACTIVE	REARING	WALKING	SNIFFING	IMMOBILE	GROOMING	HEAD MOVEMENTS	CIRCLING	BACK WALKING	SIDE WALKING
RAT FOOD (Controls)	6	15				+	+					
		20				+	+					
		25	+			+						
		30	+	+	+				+			
		35	+	+	+				+			
		40	+			+			+		+	+
		45	+			+			+		+	
		60	+			+				+		+
		75	+			+				+		
RAT FOOD W.P. (Normal)	8	15	+	+	+	+						
		20	+	+	+	+		+				
		25	+		+			+				
		30	+		+	+			+			
		35	+		+	+			+			
		40	+	+	+	+			+		+	+
		45	+		+	+			+		+	+
		60	+	+		+			+		+	+
		75	+		+	+			+	+		+
SPECIAL DIET W.P.	8	15	+	+								
		20					+					
		25	+		+	+						
		30	+		+	+			+			
		35	+		+	+			+	+		+
		40	+		+	+			+	+	+	+
		45	+	+	+	+			+	+	+	+
		60	+	+	+	+			+	+	+	+
		75	+	+	+	+			+	+	+	+

* Amphetamine was administered to the rats 3 hours after the test dose
W.P. = Wheat Protein

These activities were also of a much stronger nature than in the control groups and lasted for longer periods.

These results demonstrated that dietary wheat protein has a substantial effect on stereotyped behavior. There was an increased occurrence, especially of circling, back and side walking patters, more recorded in the "wheat-free" than in the "normal" but of earlier incidence in both than in controls, and stronger.

The results of the immunological tests on serially diluted serum and homogenates are given in Table 2. It can be seen from these results that there is a marked uptake of wheat protein into the body tissues of "wheat-free" rats. No positive reactions were recorded with brain macerates. The "wheat-free" rat serum contained three times the amount of antigenic material and the liver ten times, suggesting that transport from the intestine to the liver is not very much affected by intestinal proteolysis, but it is interesting to note that carcass content, like the serum is only three times greater suggesting much breakdown in the liver tissue.

It is also interesting to note that the content of the washed intestine in "normal" rats is twice that left in the "wheat-free" rat intestine. This is presumably the protein left in the cells, suggesting enhanced transmission by the "wheat-free" intestine.

TABLE 2
IMMUNOLOGICAL ASSAYS OF SERUM AND TISSUE ANTIGEN

Experimental Sample	Log titre (mean).	Mean± s.e.	Mean titre	p.
Serum.				
"normal"	0.8656.	0.8656±0.106	7.34.	.005
"w.p."	1.3169.	1.3169±0.080	20.74	
Liver				
"normal"	0.6021.	0.6021±0.052	4.0	.001
"w.p."	1.6440.	1.6440±0.170	44.06	
Intestine				
"normal"	2.0570.	2.0570±0.050	114.0	.001
"w.p."	1.7685.	1.7685±0.040	58.67	
Carcass				
"normal"	0.3012.	0.3012±0.061	2.0	.001
"w.p."	0.8277.	0.8277±0.062	6.73	

Conclusion

That diet may be an important factor in the pathogenesis of abnormal behavior has been demonstrated by Singh and Kay (1976), and the experiments reported herein show that drug induced abnormal behavior in rats can be influenced by dietary constituents.

Control amphetamine treated rats behaved as expected, showing the pattern of stereotyped behavior. The pattern of "normal" rats fed wheat protein differed in that these rats exhibited more head-movements and back and side walking. This behavior pattern lasted for a longer period. "Wheat-free" experimental rats presented a different pattern. The behavior was exaggerated, the animals being much more excited, and the movements of the behavior pattern much more pronounced, with some of the rats displaying aggressive tendencies.

Taylor (1978), suggested a distinctive interaction on amphetamine behavior after i.v. administration of low doses of gliadin. From the present experiments, with the knowledge that dietary proteins are transmitted from the intestinal lumen in an un-degraded form, it is not surprising that an enhanced behavior pattern of the same type was observed, as the animals had received quite a massive oral dose of wheat protein.

Turning to the immunological assay of the antigen absorbed from the intestine, it was observed that more of the wheat protein was transported from the intestine of the "wheat-free" animal. It is tentatively

concluded that these rats had transported and released more antigenically intact protein into the circulation and tissues than had the "normal" rats fed with wheat protein. It may be an immunological barrier was incomplete in animals lacking previous dietary exposure of wheat. The intestines of the "wheat-free" animals had retained less than the intestine of the "normal", suggesting a mechanism of retention of antigen in or on the intestinal walls of the latter which may be related to possible immunity.

This raises the interesting question of first contact with a foreign substance in the diet. Both the "normal" and control rats used in these experiments had been in contact with wheat protein since birth receiving daily doses. The "wheat-free" experimental rats had not. It may be "normal" and control rats were equipped with antibodies against these proteins whereas the "wheat-free" were not. Antibodies would probably be present in the form of secretory IgA forming a lining on the intestinal wall and influencing the uptake of the protein from the lumen. In the "wheat-free" rats which had not encountered the substance, the intestinal wall would either be devoid of these antibodies, or would have a much lower concentration. This in turn would offer a reduced immunological defense system and result in more protein passing from the lumen into the circulation and to the liver via the lymphatics and portal vein.

It is concluded from these experiments that wheat protein can influence the amphetamine behavior pattern in rats. After its release from the intestinal lumen the protein can be found throughout the body in an antigenically intact form. Failure to detect the protein in the brain tissue of these animals does not necessarily mean that it is absent; it may be the method of testing was not sensitive enough, Hemmings (1977) demonstrated the presence of protein in brain using isotopic assays. A radioimmunoassay method might also be more informative.

It is at least possible the change in behavior noted in this work is related to the presence of wheat proteins in the brain

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