The induction of obesity in the rat with bipiperidyl mustard

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(*Received* 5 *December* 1979 – *Accepted* 3 *June* 1980)

1. Bipiperidyl mustard produced increased adiposity in the rat without excessive weight gain or hyperphagia.
2. The results suggest a metabolic aetiology for the obesity as opposed to a disorder of food intake regulation.

Lesions in the ventromedial region of the hypothalamus were shown by Hetherington & Ranson (1940) to produce hyperphagia and obesity in the rat. The chemical agents gold thioglucose (GTG; Brecher & Waxler, 1949) and bipiperidyl mustard (BPM; Rutman *et al.* 1966) which are widely used to induce obesity in mice also produce lesions in this region of the hypothalamus (Marshall *et al.* 1955; Rutman *et al.* 1966). The use of GTG to induce obesity in the rat has proved to be unsuitable for widespread use and would be very expensive (Mayer & Marshall, 1956; Wagner & de Groot 1963). Bunyan *et al.* (1976) have shown that monosodium glutamate administered to newborn rats and mice produces obesity without hyperphagia.

Miller (1979) has recently reviewed non-genetic models of obesity and has summarized methods which produce a high proportion of obese mice, with low mortality, using monosodium glutamate or gold thioglucose, but although he mentions the use of BPM in mice he does not recommend it because it is carcinogenic and difficult to obtain. He concludes that in all the models of obesity efficiency of energy utilization is the most important factor in the aetiology of the obesity.

We now report an experiment in which obesity has been induced in the rat without mortality and inexpensively using BPM.

MATERIALS AND METHODS

Forty female Wistar rats weighing 170–200 g were housed in groups of five in large plastic cages with metal gridded floors. The animals were given FFG (M) diet (Dixon's, Ware, Dorset) and tap water *ad lib.* The animals were weighed regularly throughout the experiment and food intake was measured with due allowance made for spillage. The animals were injected intraperitoneally with approximately 0·01, 0·015 and 0·02 mg/g body-weight of BPM (Aldrich Chemical Company, Wembley, Middlesex) cyclized by incubation in borate buffer, pH 9 (0·01 M-borax/0·02 M-sodium chloride) at 37° for 1 h before injection (Rutman *et al.* 1966); control animals were injected with buffer only. The rats were killed by diethyl ether suffocation 12 weeks after injection and the final weights and nasoanal lengths were recorded. Fat content was measured by drying and Soxhlet extraction as previously reported (Rogers & Webb, 1980). Statistical analyses were performed using one-way analysis of variance, and in one instance the correlation coefficient was calculated.

0007-1145/80/3449-0306 $01.00

Table 1. *The initial and final weights (g), the weights 1 week after injection (g), weight gains (g), nasoanal lengths (mm), fat contents (g/kg) and fat free masses (g) together with food intakes (g/group per week) of female rats injected with various doses of cyclized bipiperidyl mustard (BPM) and killed 12 weeks later*

(Mean values with their standard errors for ten observations except food intakes where values are means of twelve weekly observations)

	Dose of BPM (mg/g body weight)							
	Control		0·01		0·02		0·03	
Wt (g)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial	188·1	2·5	187·3	2·2	184·4	3·2	183·8	3·0
After one week	208·3	2·2	187·4	3·6	189·1	7·7	177·5	4·3
Final	275·1	4·8	273·9	6·1	274·7	8·2	271·0	5·1
Total gain	87·0	4·3	86·6	5·4	90·9	7·0	86·2	5·1
Nasoanal length (mm)	224	1	221	2	222	2	218	1
Fat content (g/kg)	1·578	0·131	1·933	0·143	2·152	0·136	2·538	0·157
Fat-free mass (g)	231·4	3·9	221·6	6·5	213·3	9·0	202·0	4·8
Food intake (g/group per week)	1201	40	1192	60	1162	51	1082	66

There was no significant effect of treatment on initial weight, final weight, weight gain, food intake or nasoanal length. There was a significant effect of treatment on weight 1 week after injection ($P < 0.01$), fat content ($P < 0.001$) and fat-free mass ($P < 0.02$) (one-way analysis of variance).

RESULTS

There was a highly significant ($P < 0.001$) effect of BPM on body fat content (g/kg) in the rat, at the doses used, without mortality (Table 1). There was an initial period of acute toxicity, after injection, when treated animals lost or failed to gain weight and this was reflected in a significant effect of treatment on body-weight 1 week after injection ($P < 0.01$) with all experimental groups being lighter than the control group. Despite the increased final body fat content (g/kg) of the animals there was no significant effect of treatment on final body-weight, thus the increased fat content was at the expense of a significantly ($P < 0.02$) reduced fat-free mass. The reduced length of the treated groups approached but failed to reach significance ($P < 0.05$), although the animals of the highest dose group were highly significantly ($P < 0.001$) shorter than the controls using a Student's 't' test.

A proportion of the rats in each of the treated groups had fat contents (g/kg) more than two standard deviations above the control mean (20% of the lowest dose, 30% of the middle dose and 70% of the highest dose). However, there was no significant increase in the variance of the fat contents (g/kg) of the treated groups as compared to the control group suggesting that there was not a clear division of animals into responders and non-responders but rather that a complete shift in the distribution of fat contents (g/kg) resulted from treatment. This is illustrated by the observation that at the two higher doses 95% of the rats had fat contents (g/kg) above the control mean. There was a significant ($P < 0.001$) negative correlation ($r - 0.59$) between weight gain in the first week after injection (inversely related to the initial toxic effect) and the eventual fat content (g/kg). Food intake was not significantly affected by treatment; there was no suggestion of any persistent hyperphagia in treated groups.

DISCUSSION

It is clear that BPM is potentially a useful addition to the available models of obesity in the rat. Thus chemically induced obesity can be studied in the rat with the advantages this has

over mice in many experiments, particularly where serial blood samples or larger volumes of blood are required. Obesity has been produced in a high proportion of the animals, at the highest dose, cheaply, without mortality and without the use of any specialized stereotaxic apparatus or surgery.

The increased adiposity without any excessive weight gain or hyperphagia but with a reduced fat-free mass suggests a metabolic origin for the obesity. There is a diversion of available energy resources from growth (i.e. increase in lean body mass) and protein synthesis towards fat storage and this indicates a possible hormonal imbalance in the aetiology. These results are strikingly similar to those reported for GTG-obesity in mice by Rogers *et al.* (1979). They reported that a proportion of outbred mice injected with gold thioglucose showed increased fat deposition despite having normal or reduced body-weights and despite the absence of any over-all hyperphagia of the treated groups during the experimental period. The doses of BPM chosen were based on our experience of this compound in the mouse and in retrospect it would have been useful to have extended the range of doses to see if the pattern of response was similar at doses nearer to the LD_{50}. In the mouse (S. A. Jagot and G. P. Webb, unpublished observations) we have induced 100% incidence of obesity and 100% overweight with doses as low as one-third of the LD_{50}. We conclude that despite its reported carcinogenicity (Miller, 1979) BPM is a useful and under-used obesity-inducing agent which is effective in both rats and mice; we have not experienced difficulty in obtaining the compound.

S.A.J. gratefully acknowledges financial assistance from the British Council.

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Printed in Great Britain