Obesity Induced in Mice Injected Intracerebrally with 4-Nitroquinoline 1-Oxide or 4-Hydroxyaminoquinoline 1-Oxide[1] (34461)

SHUTARO YAMAMOTO, TAKEO MIZUTANI, CHOJI KANEUCHI, AND YASUHIKO SHIRASU
(Introduced by J. H. Weisburger)

The Institute of Physical and Chemical Research, Yamato-machi, Saitama-ken, Japan

The development of obesity was unexpectedly noticed in mice injected intracerebrally with the carcinogen 4-nitroquinoline 1-oxide (NQO) (1, 2) in an experiment aiming to produce brain tumors. A series of confirmatory experiments were carried out employing NQO and two related compounds, 4-hydroxyaminoquinoline 1-oxide (HAQO) and 4-aminoquinoline 1-oxide (AQO). NQO can be converted to AQO through HAO by catalytic or enzymatic reduction (3–5). HAQO is more proximate and more potent carcinogen compared with the parent NQO whereas AQO is inactive (6–8).

Materials and Methods. Female albino mice of a genetically nonobese inbred strain, ddOM, were given a single unilateral intracerebral injection of the chemicals at the age of 8 weeks. The compounds were finely ground and mixed with Tween 80 in a mortar; a suspension was prepared by gradually adding physiological saline with stirring. The concentration of Tween 80 was 0.5%. A volume of 0.02 ml of the suspension was injected. The concentrations, determined by preliminary dose finding tests, were as follows: 0.2% (0.04 mg) for NQO and AQO, and 0.05% (0.01 mg) for NQO and HAQO·HCl. Control animals were given 0.02 ml of vehicle.

The hair over the injection site was removed and the area was sterilized with tincture of iodine. Injection was made vertically into the center of the right parietal bone of mice under anesthesia with Nembutal (50 mg/kg), using a "two-step needle," commonly used for viral inoculation into the mouse brain. This special needle has a slender tip, 0.42 mm in diameter and 3.0 mm in length, connecting the rest of the needle, 0.72 mm in diameter and 11.0 mm in length. Accordingly, insertion of this needle into the cerebrum could be stopped at the depth of 3 mm. Collodion was placed over the injection site after administration of the chemicals. The animals were maintained on a diet of a commercial solid food (CMF, Oriental Co.) and water *ad libitum*.

Results and Discussion. Intracerebral injection of HAQO caused a higher death rate than did NQO. Of the mice treated with 0.05% HAQO, mortality at week 12 was 78 versus 22% after 0.2% NQO, whereas all the mice injected with 0.05% NQO survived. Administration of 0.2% AQO or of the vehicle caused no deaths. Most of the animals died within 10 days after the intracerebral injection. Symptoms of nervous disorders, such as circular movement, ataxia, and depression with gradual reduction of body weight, were more pronounced in the mice which died at a later stage.

Obesity developed only in the two groups given 0.2% NQO or 0.5% HAQO (Table I). A steep rise in body weight accompanied by hyperphagia was noticed as early as 3 days after the injection in obesity-bound mice. Almost all the obese mice could be recognized by their corpulent shape and their extraordinary body-weight gain within 4 weeks (Fig. 1 and 2). At this stage of the experiment, 40 and 37% of the mice were obese in the 0.2% NQO and 0.05% HAQO groups, respectively. Within the experimental period obese mice showed a steady increase in body weight ranging up to 60 g whereas the mean body weight of control, AQO, and 0.05% NQO groups remained in the range from 23 to 27 g. The body weight increase in the 0.05%

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TABLE I. Induction of Obesity in Mice by Derivatives of 4-Nitroquinoline 1-Oxide.

Group[a]	Conc[b] (%)	Dose (mg)	Initial no. of mice	4 Week: No. of mice surviving	4 Week: No. of obese mice	4 Week: (%)	12 Week: No. of mice surviving	12 Week: No. of obese mice	12 Week: (%)
NQO	0.2	0.04	60	50	20	40	47	21	45
	0.05	0.01	40	40	0	0	40	0	0
HAQO	0.05	0.01	98	35	13	37	22	8	36
AQO	0.2	0.04	39	39	0	0	39	0	0
Control			40	40	0	0	40	0	0

[a] NQO, 4-nitroquinoline 1-oxide; HAQO, 4-hydroxyaminoquinoline 1-oxide; AQO, 4-aminoquinoline 1-oxide.

[b] A volume of 0.02 ml was injected.

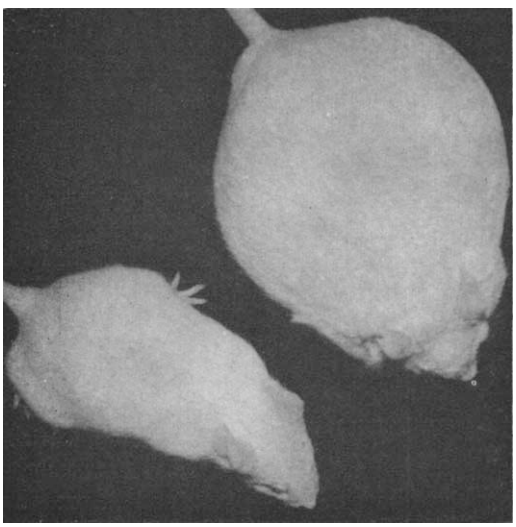

FIG. 1. An obese mouse obtained after intracerebral injection with NQO, in contrast with a control mouse.

HAQO groups was significantly higher than that of the 0.2% NQO groups.

Autopsy of the obese animals that died during the experimental period revealed marked fat deposition throughout the body, especially in the abdominal cavity. Histopathologic studies disclosed necrosis in the nerve cells in the dorsolateral region of the Ammon's horn in the injected side. The nerve cells in the same area of the Ammon's horn of the opposite hemisphere were also affected.

In order to confirm hyperphagia, food and water intake along with the body weight were measured daily in mice placed in individual metabolism cages. Obesity was induced in 6 of 20 mice injected intracerebrally with NQO. A conspicuous hyperphagia followed by a steep increase of the body weight was clearly demonstrated in obese mice (Fig. 3). The water intake showed a similar elevated pattern.

A dietary restriction experiment was carried out in 4 obese mice produced by injection in NQO. After free feeding for 7 days in the metabolism cages, the daily food supply was restricted to 4.0 g, the average intake in the control animals. Drinking water was given *ad libitum*. Free feeding was resumed after 14 days. The obese mice lost about 28% of their body weight during the restriction period (Fig. 4). Return to free feeding caused a steep rise in food intake followed by an increase in body weight. The water intake showed the same trend as the food consumption. The dietary restriction experiment may indicate a persistent injury to the dietary regulatory system in these obese mice.

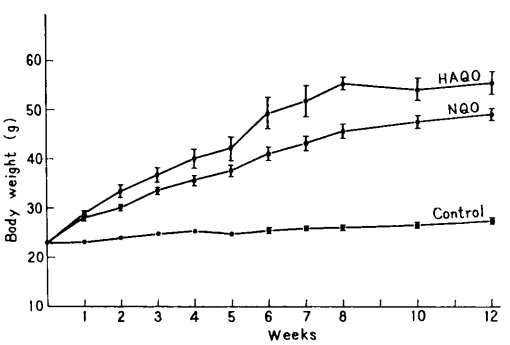

FIG. 2. Body weight changes of the obese mice seen after intracerebral injection with NQO and HAQO. The data shown are mean values ± standard error (see Table I for the numbers of mice).

Similar treatment with the following five compounds at the maximum levels using 40 or 50 mice for trial failed to produce obesity: 2 are noncarcinogenic analogs of NQO (9), 4-nitropyridine 1-oxide (1.5%, 0.3 mg), 3-methyl-4-nitroquinoline 1-oxide (0.2%, 0.04 mg), and 4 are carcinogens of different types, *N*-nitrosomethylurea (2%, 0.4 mg), 3,4-benzpyrene (2%, 0.4 mg), *N*-hydroxy-*N*-2-fluorenylacetamide (5%, 1.0 mg) and β-propiolactone (2%, 0.4 mg). From these findings, it appears that NQO and HAQO have a peculiar ability to produce obesity when injected into the mouse brain.

Obesity has been induced by electrolytic destruction of the ventromedial nuclei of the hypothalamus or by administration of gold thioglucose (10, 11). Further studies are now in progress to investigate the relationship between the injury in the dorsolateral region of the Ammon's horn and these satiety centers.

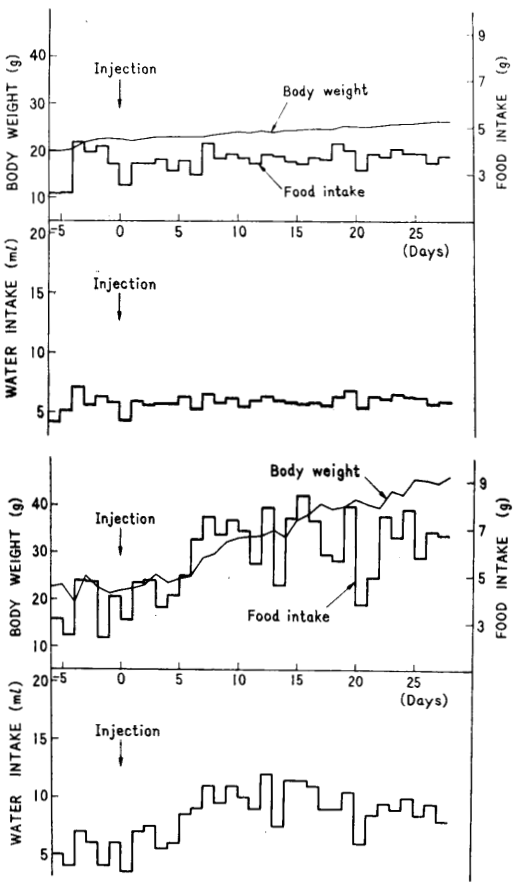

FIG. 3. Food and water intake during the development of obesity. The averages of 6 controls (top); and 4 obese mice (bottom).

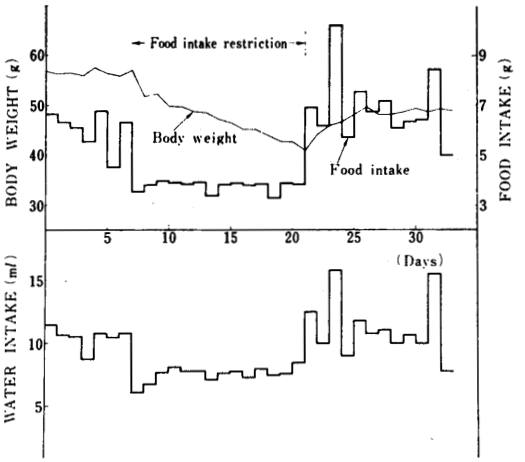

FIG. 4. Food intake restriction experiment in obese mice. The data are averages of 3 mice.

Summary. Obesity was induced in 45 and 36% of mice by intracerebral injection with the carcinogens 4-nitroquinoline 1-oxide (NQO) and 4-hydroxyaminoquinoline 1-oxide (HAQO), respectively, but not with the related noncarcinogens, 4-aminoquinoline 1-oxide, 3-methyl-4-nitroquinoline 1-oxide, and 4-nitropyridine 1-oxide. Food and water intake were markedly increased in the obese mice. While dietary restriction decreased the body weight, release of the restriction caused a conspicuous increase of food intake followed by a gain in body weight. No obesity could be induced with the following 4 local carcinogens: 3, 4-benzpyrene, β-propiolactone, *N*-nitrosomethylurea and *N*-hydroxy-*N*-2-fluorenylactamide.

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