binding interaction translates functionally to antagonism of the Bezold–Jarisch reflex in vivo. Thus in mice, renzapride, zacopride, and BIMU-8 antagonized the 5-$HT_3$ receptor-mediated bradycardic response to serotonin with $ED_{50}$ values of 0.25, 0.005, and 0.01 mg/kg ip. By contrast, the pyrrolizidine **1B** exhibited an $ED_{50}$ of 3.0 mg/kg ip, consistent with the modest $K_i$ of 152 nM in the 5-$HT_3$ binding assay.

In conclusion, the substituted pyrrolizidines **1** afford a novel series of potent and selective 5-$HT_4$ agonists. **1B** is the most potent and selective agent yet reported for agonism at this newly identified serotonin receptor. While exhibiting potency ($ED_{50}$ = 23 nM) similar to serotonin in the 5-$HT_4$ rat esophagus model, it is only modestly active at the 5-$HT_3$ receptor ($K_i$ = 152 nM) compared to zacopride (0.23 nM), renzapride (5.3 nM), and BIMU-8 (7.0 nM). Moreover, unlike metoclopramide and cisapride, **1B** exhibits little affinity ($IC_{50}$ > 10000 nM) for dopamine $D_2$, 5-$HT_2$, or $\alpha_1$ adrenergic receptors. The in vitro 5-$HT_4$ agonist property of **1B** correlates well with potent gastrointestinal prokinetic activity in vivo, the results of which will be reported in due course. **1B** will undoubtedly be a useful agent for probing the role of 5-$HT_4$ receptors in various CNS and gastrointestinal diseases.

**Supplementary Material Available:** Physical data for intermediates and final products (7 pages). Ordering information is given on any current masthead page.

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*Received November 25, 1991*

## Bis(maltolato)oxovanadium(IV) Is a Potent Insulin Mimic

Diabetes is a mammalian condition in which the amount of glucose in the blood plasma is abnormally high.[3] The condition can be acutely life-threatening and, in addition, patients with diabetes suffer from a number of secondary complications, for example, atherosclerosis, microangiopathy, renal disease and failure, cardiac disease, and diabetic retinopathy and other ocular disorders including blindness. Millions of sufferers control diabetes by daily insulin administration and/or diet. Insulin replacement is the easiest method of controlling chronic diabetes; however, insulin is not orally active and must be taken by injection. There is great interest in orally active insulin mimics, particularly vanadium compounds.[4]

In the late 1970s sodium orthovanadate ($Na_3VO_4$, vanadium(V)) was found by Cantley and co-workers to be a potent inhibitor of $Na^+$,$K^+$-ATPase.[5] Shortly thereafter, the same group showed that vanadate taken up by red blood cells was reduced to vanadium(IV) (the vanadyl ion $[V{=}O]^{2+}$) in the cytoplasm.[6] Since then, there has been significant research on the glycosidic function of vanadium (mostly as vanadate) because cardiac glycosides are known to inhibit specifically $Na^+$,$K^+$-ATPase. A natural outgrowth of this has been the study of vanadium and diabetes.[4] The insulin-like effect of vanadate ion ($VO_4^{3-}$) has been known since 1980[7] and is currently under active investigation. The same group showed in the same paper that the insulin-like stimulation of glucose oxidation in rat adipocytes was due to the vanadyl ion. Our group demonstrated that vanadate, administered in drinking water, reduced elevated blood glucose to normal and restored depressed cardiac performance in rats made diabetic with streptozocin (STZ) in 1985.[8] Interest in the insulin-mimicking effect of vanadate and vanadyl has burgeoned since Sakurai and co-workers showed that vanadate is reduced in vivo to vanadyl.[9] Significant drawbacks to vanadate are that it is poorly absorbed from the gastrointestinal tract into the blood and that it is toxic; administered concentrations are, therefore, close to the toxic level in order to manifest the insulin-mimicking effect in animals.

Subsequent work by McNeill et al.[10-14] has shown that vanadyl administered orally as aqueous vanadyl sulfate $[VO(H_2O)_5]SO_4{\cdot}(H_2O)_x$ will also lower blood glucose and blood lipids in STZ diabetic rats and will prevent secondary complications of diabetes such as cataracts and cardiac dysfunction. Vanadyl sulfate is less toxic than vanadate but is also poorly absorbed. There have been

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0022-2623/92/1835-1489$03.00/0 

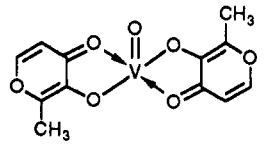
**Table I.** Parameters Determined for the Four Groups at Week 4[a-c]

| parameter | C | CT | D | DT |
|---|---|---|---|---|
| weight (g) | 404 ± 12 | 391 ± 7 | 349 ± 7[d] | 311 ± 1[d,e] |
| food (g rat$^{-1}$ day$^{-1}$) | 28.3 ± 1 | 28.8 ± 1.6 | 63.5 ± 1.3[d] | 37.9 ± 0.9[d,e] |
| fluid (mL rat$^{-1}$ day$^{-1}$) | 59 ± 3 | 43 ± 2 | 264 ± 18[d] | 52 ± 7 |
| plasma glucose (mM) | 4.0 ± 0.1 | 4.1 ± 0.1 | 13.9 ± 0.2[d] | 7 ± 0.8[d,e] |
| plasma insulin ($\mu$Units mL$^{-1}$) | 35.6 ± 1.3 | 21.6 ± 1.2[d] | 22.0 ± 2.6[d] | 21.9 ± 1.6[d] |
| plasma cholesterol (mM) | 1.4 ± 0.01 | 1.7 ± 0.01 | 2.4 ± 0.7[d] | 1.6 ± 0.1 |
| plasma triglyceride (mM) | 1.8 ± 0.01 | 2.1 ± 0.2 | 2.7 ± 0.7 | 1.9 ± 0.2 |
| dose of I (mmol kg$^{-1}$) | | 0.19 ± 0.01[g] | | 0.37 ± 0.03[f,h] |

[a] Control (C, $n$ = 8), control-treated (CT, $n$ = 11), diabetic (D, $n$ = 11), diabetic-treated (DT, $n$ = 12). [b] Mean ± SEM. [c] Statistical differences between the means of the various groups were evaluated using ANOVA followed by a Newman-Keuls test ($p < 0.05$ significant). [d] $p < 0.05$, different from C. [e] $p < 0.05$, different from D. [f] $p < 0.05$, different from CT. [g] 59.2 ± 2.9 mg kg$^{-1}$. [h] 113.9 ± 6.4 mg kg$^{-1}$.

only two attempts (to our knowledge) to chemically modify the biological uptake of vanadium by changing the chemical form in which it is supplied from either vanadate $VO_4^{3-}$ or vanadyl sulfate (the latter has been used because the active form of vanadium may well be the vanadyl ion). Work on vanadium peroxide compositions as insulin mimics involved in vitro studies of vanadate and peroxide coadministered and the nature of the administered, or for that matter the effective, chemical species is quite unknown.[15-17] A vanadyl cysteine compound has also been proposed for the oral treatment of diabetes.[18]

Bis(maltolato)oxovanadium(IV) ($C_{12}H_{10}O_7V$, I) is prepared nearly quantitatively (>90% yield) in water by



I

combining maltol (3-hydroxy-2-methyl-4-pyrone) and vanadyl sulfate (2:1), raising the pH of the solution to 8.5, refluxing overnight, and collecting the deep purple-green (birefringent) compound which precipitates upon cooling.[19] The direct electrochemical preparation of I was reported in 1978 although the compound was incompletely characterized;[21] its electron paramagnetic resonance spectrum was reported in 1972[20] and again in 1987.[22] Alkoxooxovanadium(V) derivatives of the maltolato anion (ma$^-$) (VO(OR)ma$_2$) have been known since the 1960s.[23]

The effectiveness of I as an oral hypoglycemic agent in the treatment of diabetes mellitus was assessed in male Wistar rats. Forty two rats were randomly divided into four groups: control (C, $n$ = 8), control-treated (CT, $n$ = 11), diabetic (D, $n$ = 11), and diabetic-treated (DT, $n$ = 12). The diabetic state was induced by the intravenous administration of streptozocin (STZ, from Sigma) at 60 mg kg$^{-1}$ in 0.9% NaCl via the tail vein. The control group received an equivalent volume of isotonic saline only. The rats were housed two or three per cage on a 12 h light/dark schedule and had free access to food and drinking water. The control and diabetic groups received tap water to drink, whereas the control-treated and diabetic-treated groups received compound I dissolved in tap water (1.58 mM). Daily, each animal was weighed and average food and fluid consumption values were determined based on the amount consumed per 24 h per cage divided by the number of animals in each cage. The data presented in Table I represent values obtained at 4 weeks after treatment was initiated. Blood was collected from nonfasted rats at week 4 by nicking the tip of the tail and expressing sufficient volumes of blood into heparinized capillary tubes to determine plasma glucose, insulin, cholesterol, and triglyceride levels in each animal. The blood was centrifuged at 20000$g$ for 10 min at 4 °C. The plasma was collected and stored at −70 °C until assays were conducted.[24]

After 4 weeks of treatment with compound I, there was a reduction in plasma glucose values in the diabetic-treated group to euglycemic levels (≤9 mM). While the diabetic-treated glucose level of 7 ± 0.8 mM was significantly higher than those for both the control groups, the level was within the acceptable range for normal plasma glucose values. The control-treated group was not different from the control group.

Among the earliest symptoms of diabetes mellitus are polyphagia and polydipsia. The diabetic-treated group demonstrated a greater reduction in body weight gain than the diabetic group. The daily food consumption for the diabetic-treated group was reduced by about 40% with respect to the diabetic group and was about 25% higher than for both the control groups. The decrease in body

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$$\frac{[\text{concentration (mmol mL}^{-1})] \times [\text{fluid consumed (mL)}]}{\text{rat weight (kg)}}$$

weight gain accompanying compound I treatment in the diabetic–treated group is likely due to a decrease in food intake and to the fact that diabetes was not totally controlled in some animals. The polydipsia observed in diabetes occurs in response to dehydration from polyuria. This effect was observed in the diabetic group which consumed approximately 5 times more fluid on a daily basis than any other experimental group while there was no difference between the control fluid consumption levels with respect to either of the treated groups. The normalization of fluid consumption in the diabetic–treated group may thus reflect the relatively normal glucose levels and the elimination of osmotic diuresis. Vanadium may have direct effects on food and fluid intake in both control and diabetic animals. Previous experiments in our laboratories (and in those of others) have demonstrated that the administration of vanadate or vanadyl can decrease fluid and food intake to less than control values.[8,10] In the present study, there was a trend toward a decrease in fluid intake in the control treated group, but this was not significant.

A reduction in plasma insulin values to diabetic levels was the only parameter which was different between the control and the control–treated groups. This may indicate that compound I reduces the level of insulin required to maintain the euglycemic state. This observation has also been reported with vanadate treatment.[8,10] The decrease in insulin cannot be attributed to a decline in food and fluid levels, since there was no difference in consumption values between the two groups. The insulin values for the two diabetic groups were the same and were not different from the control–treated group. Thus the effect of compound I is not due to insulin release. The dose of I is dependent on both fluid consumption and body weight. The higher dose of compound I received by the diabetic–treated group can be attributed both to a reduction in body weight and to a slight increase in fluid intake over the control–treated group.

At week 4, the cholesterol levels in the diabetic group were elevated compared with those in the control group. The increase in fatty acid metabolism is typical in diabetes and has been associated with the development of atherosclerosis in diabetic patients, and with an increased risk of coronary heart disease.[25] Treatment with I reduced cholesterol levels to control values in the diabetic–treated group as has been previously shown in our laboratory using vanadyl sulfate.[10] There was no observed effect on lipid levels in the control–treated group with respect to the control group. Triglyceride levels were the same in all of the experimental groups at this point.

In summary, compound I is a discrete easily-prepared vanadium compound which normalizes glucose and lipid values and restores food and fluid intake to control levels in diabetic rats without an increase in insulin levels. It does not normalize weight gain. In the control treated animals, plasma insulin levels were reduced. These results are similar to previous reports from our laboratory using vanadyl sulfate to treat diabetic animals.[10] Compound I was specifically designed to be well-absorbed across the gastrointestinal membranes. The dose of compound I administered to the diabetic–treated group in this experiment was 0.37 mmol $kg^{-1}$; this is compared with a dose of 0.6 mmol $kg^{-1}$ of vanadyl sulfate trihydrate at the same time point in previous work from this laboratory.[10] The potency of compound I is about 50% greater than that of vanadyl sulfate. (Direct comparisons cannot be made between two different groups of rats; however, further comparison studies are ongoing.) In those past experiments, the reduction in blood glucose was observed to occur after 1–2 weeks of treatment; however, with I blood glucose levels were significantly reduced within 24 h of the initiation of treatment. In addition, toxicity, notably dehydration due to failure to drink, which is sometimes seen with vanadyl sulfate, was not observed with compound I. The compound may find application as either a treatment for diabetes or an appetite suppressant, or both. Further studies of the mechanism of action of I, its aqueous coordination chemistry, and the development of other analogues are in progress.

**Acknowledgment** is made for operating grants to the Canadian Diabetes Association (J.H.M.) and to the Natural Sciences and Engineering Research Council of Canada (C.O.), and to Mary Battell for technical assistance.

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