MOLECULAR PHARMACOLOGY, 18, 402–405

Progesterone Derivatives that Bind to the Digitalis Receptor: Structure–Activity Relationships

RYUNG-SOON (SONG) KIM,*,[1] FRANK S. LABELLA,*,[2] HILDA ZUNZA,†,[3] FEDERIKA ZUNZA† AND JOHN F. TEMPLETON†

* *Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada, R3E 0W3, and † Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2*

Received March 14, 1980; Accepted July 2, 1980

SUMMARY

KIM, R. S., F. S. LABELLA, H. ZUNZA, F. ZUNZA AND J. F. TEMPLETON. Progesterone derivatives that bind to the digitalis receptor: Structure–activity relationships. *Mol. Pharmacol.* **18:** 402–405 (1980).

Certain progesterone derivatives compete for binding in a cardiac glycoside radioreceptor assay. Systematic examination of structurally modified progesterones shows that substitution and/or unsaturation in the B ring of the steroid nucleus and the presence of 17α-acetoxy are important determinants of binding potency. Although 17α-acetoxyprogesterone itself is very weak, substitution of that steroid at C-6 by either 6α-methyl, 6α-chloro, or 6α-bromo groups markedly enhanced receptor binding. This effect may be due to the long-range influence of 17α-acetate plus the steric strain introduced by 6α-substitution which lead to inversion of the A ring to a digitalis-like conformation. Substitution plus unsaturation at C-6 appear to be more important than either alone, as indicated by the highly potent chlormadinone acetate, bromadinone acetate, and megestrol acetate. Steric rather than electronic effects are operative in the potency enhancing effects of C-6 substituents. Structural modifications at the C-1 and C-3 positions led to minimal effects on binding to the digitalis receptor.

INTRODUCTION

In spite of the extensive use of cardiac glycosides for the management of cardiovascular diseases, the difficulties and risks of this therapy have not been overcome. This is because of the narrow therapeutic spectrum (1, 2) as well as the wide differences in patient response to this class of compounds (3–5). Although the search for natural or synthetic substances with improved therapeutic properties has been pursued extensively, it has thus far not produced satisfactory results. Prerequisites for success are knowledge of the structure–activity relationships of cardioactive molecules which might form the basis of dissociating therapeutic from toxic effects. It is possible to separate these effects if there are two different mechanisms or two different receptor sites.

In a previous study from this laboratory, certain derivatives of progesterone were found to specifically inhibit [^{3}H]ouabain binding to dog heart (6). Chlormadinone acetate (CMA),[4] the most potent of the steroids tested in the binding assay, is a widely used progestin and a component of some oral contraceptives. We have also demonstrated (7) that CMA possesses certain ouabain-like properties, i.e., inhibition of (Na^+ + K^+)-ATPase (ATP phosphohydrolase; EC 3.6.1.3) and the sodium pump in the skeletal and cardiac muscle. In a subsequent study (8) CMA was shown to inhibit, noncompetitively with respect to ATP and competitively with respect to KCl, (Na^+ + K^+)-ATPase isolated from lamb kidney (IC_{50}, 3 μM), cat heart (6 μM), and guinea pig (30 μM). Although CMA seems to act at the same site on the enzyme as ouabain (8), it neither enhanced cardiac contractility nor antagonized the positive inotropic effect of ouabain, thus indicating that ATPase inhibition can be dissociated from enhanced contractility of cardiac muscle. However, the possibility that the lack of activity of CMA may be due to its limited solubility and/or partition into lipid compartments of the muscle under the experimental conditions or to the presence of distinct receptor

Supported by the Manitoba Heart Foundation, the Sellers Foundation, and the Richardson Foundation.

[1] Postdoctoral Fellow of the Canadian Heart Foundation.

[2] MRC Career Investigator.

[3] On leave from Instituto de Quimica, Universidad de Concepción, Casilla 3-C, Concepción, Chile.

[4] Abbreviations used: CMA, 17 α-acetoxy-6-chloropregna-4,6-dien-3-one (chlormadinone acetate); (Na^+ + K^+)-ATPase, Na^+- and K^+-stimulated adenosine triphosphatase; RRA, radioreceptor assay; IC_{50}, molar concentration for 50% inhibition of specific binding.

0026-895X/80/060402-04$02.00/0

sites for CMA and ouabain in intact tissues has not been ruled out.

Elucidation of the structure–activity relationships of progesterone derivatives in relation to digitalis receptor binding, sodium pump inhibition, and cardiotonic (inotropic) activity may lead to a better understanding of the mechanisms of inotropic and toxic actions of cardiac drugs and the ultimate discovery of agents exerting only inotropic actions. Furthermore, the possibility exists that endogenous progestins serve as physiological ligands for the digitalis receptor.

To gain a greater insight into the molecular specifications for binding activity in the [^{3}H]ouabain radioreceptor assay (RRA), as well as other biological activities, derivatives related to 17α-hydroxy-progesterone were examined, and the results are presented in this report.

MATERIALS AND METHODS

The [^{3}H]ouabain RRA was based on the original method by Matsui and Schwartz (9) and was slightly modified as published previously (6, 7). Protein was estimated by the method of Lowry *et al.* (10). 17α-Acetoxy-6-chloropregna-4,6-dien-3-one (CMA; **V**) was obtained from Lilly Research Laboratories, Indianapolis, Ind., and its derivatives (**VIa** and **VIb**) from Ayerst Research Laboratories, Saint-Laurent, P.Q.; 17α-hydroxy-1α,2α-cycloproprano-6-chloro-4,6-pregnadiene-3,20-dione (cyproterone; **IXa**) and cyproterone acetate (**IXb**) from Schering A.G., Berlin, through the courtesy of Pentagon Laboratories, Ltd., Montreal, P.Q.; 6-chloro-17α-acetoxy-pregna-1,4,6-trien-3-one (delmadinone acetate; **VII**) from Syntex Research Laboratories, Palo Alto, Calif.; 4,6-dichloro-17α-hydroxypregna-4,6-diene-3,20-dione acetate (**VIII**) from Hoffman–La Roche Inc., Nutley, N.J.; and 6-chloro-16-methylene-17α-acetoxy-4,6-pregnadiene-3,20-dione (**XVII**) from Schering Corp., Bloomfield, N.J. 6-Methyl-17α-acetoxypregna-4,6-dien-3-one (megestrol acetate; **XI**; Megace–Bristol Laboratories of Canada, Candiac, P.Q.) was extracted from tablets and purified by recrystallization. Progesterone (**Ia**), 17α-hydroxyprogesterone (**Ib**), and 6α-methyl-17α-acetoxyprogesterone (medroxyprogesterone acetate; **Xb**) were obtained from Sigma Chemical Co., St. Louis, Mo., and 17α-acetoxyprogesterone (**Ic**), 17α-caproxyprogesterone (**Id**), and 6α-methylpregna-3,16-diene-3,20-dione (**XVIII**) from Steraloids, Inc., Wilton, N.H.

The following compounds were synthesized by standard methods: 17α-acetoxy-pregna-4,6-diene-3,20-dione (**II**), 17α-acetoxypregna-1,4-diene-3,20-dione (**III**), and 17α-acetoxypregna-1,4,6-triene-3,20-dione (**IV**) (11); medroxyprogesterone (**Xa**) from alkaline hydrolysis of the medroxyprogesterone acetate, **Xb**; 6α/6β-bromo-17α-acetoxypregna-4-ene-3,20-dione (**XIII** and **XIV**) and 6α/6β-chloro-17α-acetoxypregna-4-ene-3,20-dione (**XV** and **XVI**) (12); and 17α-acetoxy-6-bromopregna-4,6-dien-3-one (bromadinone acetate; **XII**), m.p. 189–191°C, m/z 448/450 (M^+), by t-butyl chromate oxidation (13) of 17α-acetoxy-3-ethoxypregna-3,5-dien-20-one (14). A sample of 17α-methyl-progesterone (**Ie**) was supplied by Dr. G. R. Duncan, Faculty of Pharmacy, University of Toronto, Ontario.

RESULTS AND DISCUSSION

Our previous study (7) on structure–activity relationships was extended to several new progesterone derivatives in the [^{3}H]ouabain binding assay. Figure 1 shows a list of compounds and their IC_{50} values, which serve as indices of comparative affinity to the receptor. Relative potencies are expressed relative to chlormadinone acetate (**V**; IC_{50}, 2 μM), the most potent pregnane in the [^{3}H]ouabain RRA assigned a value of 100. The concentration-displacement curves of progesterone derivatives in the [^{3}H]ouabain RRA are shown in Fig. 2. The IC_{50} ranges for the poorly water-soluble compounds (**II**, **IXa**, and **Xa**) were obtained by extending the straight-line portion of their respective concentration–displacement curves where they were completely soluble. Systematic structural modifications of progesterone derivatives have led to the following generalizations with respect to ouabain receptor binding.

C-6 position. Progesterone (**Ia**), 17α-hydroxyprogesterone (**Ib**), 17α-acetoxyprogesterone (**Ic**), and 17α-caproxyprogesterone (**Id**) had little or no activity ($IC_{50} > 1000$ μM). Whereas 17α-acetoxyprogesterone (**Ic**) itself is essentially inactive in the [^{3}H]ouabain binding assay, substitution at C-6 leads to active analogues: 6α-methyl-17α-acetoxyprogesterone (**Xb**; IC_{50}, 16 μM), 6α-chloro-17α-acetoxyprogesterone (**XV**; IC_{50}, 10.5 μM), and 6α-bromo-17α-acetoxyprogesterone (**XIII**; IC_{50}, 16 μM). Substitution at the C-6 position has also been known to have a dominant and controlling effect on the dimensions of progestational activity among the 17α-acetoxyprogesterones and their 16-alkylated counterparts (15). Substitution of CMA to give the 16-methylene derivative (**XVII**; IC_{50}, 4.4 μM), a change associated with increased progestational activity (15), has little effect on binding to the ouabain receptor.

X-Ray crystallographic studies show that the addition of a 17α-acetoxy substituent to the progesterone molecule restricts the flexibility of the C-17 side chain and has a long-range conformational effect on the A-ring, causing it to adopt a perfect 1α-sofa conformation (16). Further, the addition of a 6α-methyl substituent causes a greater change in the A-ring conformation, which now becomes a 1β-2α-inverted half-chair (17). That this latter conformational change which now resembles digitalis glycosides may be associated with the increased activity of the 6α-substituted derivative is indicated by the inactivity of 6β-bromo (**XIV**; $IC_{50} > 1000$ μM)- and 6β-chloro-17α-acetoxyprogesterone (**XVI**; $IC_{50} > 1000$ μM), which would not be expected to affect the conformation of the A ring in the same way.

Substitution and/or unsaturation in the B ring of the steroid nucleus appears to be a major determinant for binding of progestins to the ouabain receptor, the combination of C-6 substitution and unsaturation being more important than either factor alone in all cases observed. The effect of unsaturation in the B ring enhances the activity somewhat as 17α-acetoxy-pregna-4,6-dien-3-one (**II**; IC_{50} 50, μM) has weak activity, whereas substitution at C-6 on the double bond gives CMA (**V**), the most potent compound tested. Similarly, bromadinone acetate (**XII**; IC_{50}, 2 μM) and megestrol acetate (**XI**; IC_{50}, 5.6

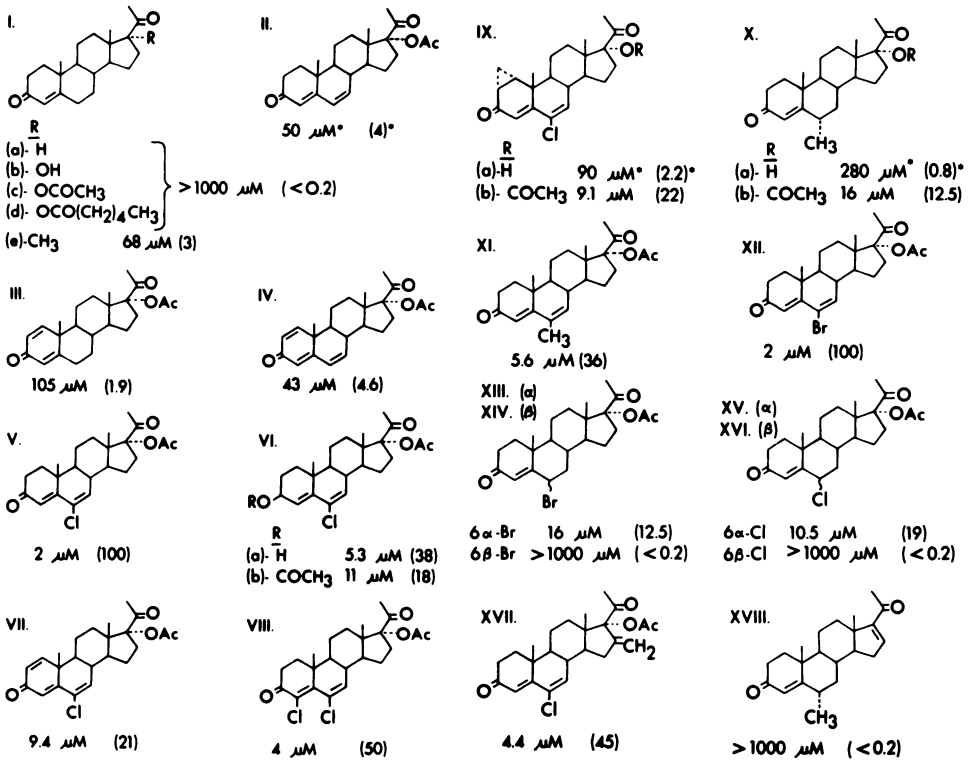


FIG. 1. *Structure and potency of progesterone derivatives in the [^{3}H]ouabain radioreceptor assay*

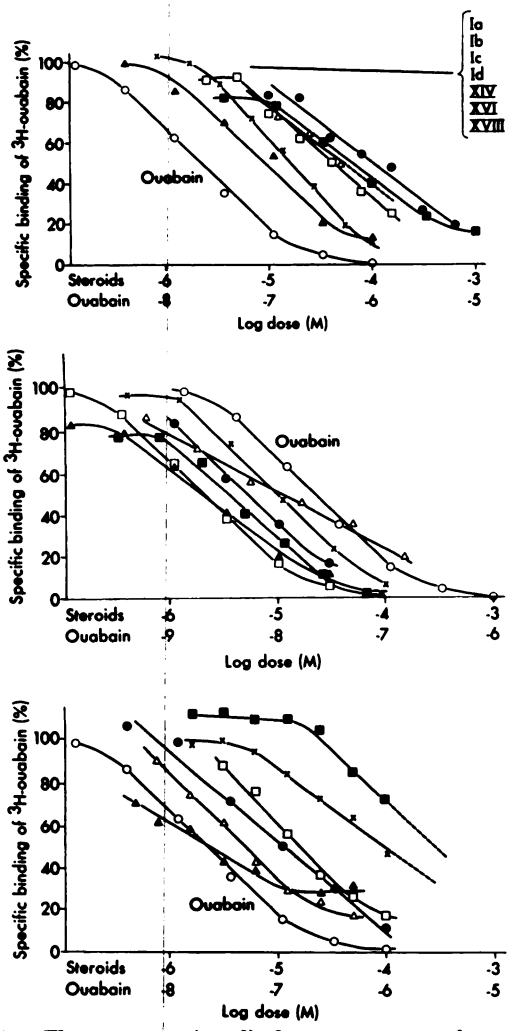
Varying amounts of competing steroids were added in 10 μl ethanol and incubated in 50 mM Tris–HCl buffer (pH 7.4) containing 150 mM NaCl, 1 mM EDTA, 1.25 mM $MgCl_2$, 1.25 mM ATP (freshly added), 9 nM [^{3}H]ouabain, and the total particulate fraction from 12.5 mg dog heart (1.7 mg protein) for 60 min at 37°C in a total volume of 1 ml. Ouabain standards and control tubes also contained 10 μl ethanol. Specific binding of [^{3}H]ouabain represents the difference in membrane-bound counts in the presence and in the absence of 1.25 mM ATP. The IC_{50} values and the relative potency (in parentheses) are indicated under the structure. The IC_{50} values, determined from the concentration–displacement curves (Figs. 2–4), are means of three to five separate assays and are compared with those of CMA (IC_{50}, 1–3 μM) and unlabeled ouabain (16–25 nM). The relative potencies relate to CMA, which is assigned a value of 100. (*) Extrapolated value.

μM) are highly active. Substitution of a second chlorine atom on the unsaturated system at C-4 to give 4,6-dichloro-17α-acetoxypregna-4,6-dien-3-one (VIII, IC_{50}, 4 μM) does not further increase activity. These results also demonstrate the importance of substitution at C-6 and suggest that a steric rather than an electronic effect is operative.

C-1 position. Introduction of unsaturation into the steroid A ring at C-1 also enhances activity. 17α-Acetoxypregna-1,4-dien-3-one (III; IC_{50}, 105 μM) and 17α-acetoxypregna-1,4,6-trien-3-one (IV; IC_{50}, 43 μM) show measurable activity, whereas the parent 17α-acetoxyprogesterone (Ic) is inactive. The conformational effect of the introduction of a double bond at C-1 is to flatten the β face approximating the inversion of the A ring as in 6α-methyl-17α-acetoxyprogesterone (X) (17). Progestational potency has been reported to be enhanced by the introduction of unsaturation at C-1 (18). Although the effect of unsaturation at C-1 appears to enhance the activity to some degree, this effect is not observed in the compounds with substituents at the unsaturated C-6 position. Thus, delmadinone acetate (VII; IC_{50}, 9.4 μM) showed a slight decrease in activity with respect to CMA (V), as is also the case with cyproterone acetate (IXb; IC_{50}, 9 μM), the A ring of which has an analogous stereochemistry to the inverted A ring of 6α-methyl-17α-acetoxyprogesterone (X) (19).

C-3 position. The nature of the substituent at C-3 appears to be less important for [^{3}H]ouabain binding activity. This is demonstrated by the relatively small change in activity observed by the C-3 derivative of CMA (V). Reduction of the C-3 carbonyl group of CMA to the 3β-alcohol (VIa; IC_{50}, 5 μM) and conversion to the 3β-acetate (VIb; IC_{50}, 11 μM) diminish but do not abolish activity.

C-17 position. All of the progesterone derivatives investigated so far which show high activity have a 17α-acetoxy group. Comparison of compounds where the 17α-acetoxy function is substituted by the 17α-hydroxyl function shows that activity is considerably diminished as in 6α-methyl-17α-acetoxyprogesterone (Xb; IC_{50}, 16 μM) and 6α-methyl-17α-hydroxyprogesterone (Xa; IC_{50}, 280 μM) or cyproterone acetate (IXb; IC_{50}, 9 μM and cyproterone (IXa; IC_{50}, 90 μM). The presence of the 17α-hydroxy group does not cause the conformational changes induced by the 17α-acetoxy group (20). Introduction of a C-16 double bond (6α-methylpregna-3,16-diene-3,20-dione, XVIII; $IC_{50} > 1000$ μM) destroys activity. Addition of a 17α-methyl instead of the 17α-acetoxy group to give 17α-methylprogesterone (Ie; IC_{50}, 68 μM) yielded an ac-



FIGS. 2–4. *The concentration–displacement curves of progesterone derivatives in the [^{3}H]ouabain radioreceptor assay*

The test compounds are represented by the Roman numerals, and their chemical structures are shown in Fig. 1. Fig. 2. Ie (■), II (△), III (●), IV (□), IXb (▲), Xb (×). Fig. 3. V (□), VIa (●), VIb (△), VII (×), XII (▲), XVII (■). Fig. 4. VIII (▲), IXa (×), Xa (■), XI (△), XIII (□), XV (●).

tive derivative. In a recent study on the structure–activity relationships of the digitalis genin, it was shown that there exists a close correlation between the orientation of the C-17 side-chain carbonyl oxygen in the digitalis genins and their analogues and (Na^+ + K^+)-ATPase inhibiting activity (21). Similar analysis of the position of the carbonyl oxygen in the 17α-acetate and other C-17 functional groups may resolve the importance of this region in relation to [^{3}H]ouabain receptor binding activity.

REFERENCES

1. Diederen, W. and R. Kadatz. Quantitative Untersuchungen der Therapeutischen Breite von Herzglycosiden. *Arztl. Forsch.* **24**: 149–155 (1970).
2. Kimble, M. A. and R. M. Elenbaas. Congestive heart failure. *J. Amer. Pharmacol. Assoc.* **14**: 362–375 (1974).
3. Amer. Pharmacol. Assoc. Cardiac glycoside therapy, in *Evaluations of Drug Interactions*. Amer. Pharmacol. Assoc., Washington, D.C., 1st ed., 295 (1973).
4. Moe, G. K. and A. E. Farah. In *The Pharmacological Basis of Therapeutics* (L. S. Goodman and A. Gillman, eds.). Macmillan, New York, 5th ed., 653 (1975).
5. Repke, K. Biochemie und Klinik der Digitalis. *Internist* (*Berlin*) **7**: 418–426 (1966).
6. Chow, E., R. S. Kim, G. Queen and F. S. LaBella. Ouabain receptor binding of hydroxyprogesterone derivatives. *Brit. J. Pharmacol.* **67**: 345–352 (1979).
7. LaBella, F. S., I. Bihler and R. S. Kim. Progesterone derivatives binds to cardiac ouabain receptor and shows dissociation between sodium pump inhibition and increased contractile force. *Nature* **278**: 571–573 (1979).
8. Wehling, M., K. R. Whitmer, A. Schwartz, G. Grupp, I. Grupp, E. T. Wallick, I. Bihler and F. S. LaBella. Interaction of chlormadinone acetate with ouabain binding site of Na, K-ATPase. *Fed. Proc.* **39**: 626 (1980).
9. Matsui, H., and A. Schwartz. Mechanism of cardiac glycoside inhibition of the Na^+,K^+-dependent ATPase from cardiac tissue. *Biochim. Biophys. Acta* **151**: 655–663 (1968).
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275 (1951).
11. Fried, J. and J. A. Edwards (eds.). *Organic Reactions in Steroid Chemistry, Vol. 1*. van Nostrand Reinhold, New York, 308–312 (1972).
12. Ringold, H. J., E. Batres, A. Bowers, J. Edwards and J. Zderic. Steroids. CXXVII. 6-Halo-progestational agents. *J. Amer. Chem. Soc.* **81**: 3485–3486 (1959).
13. Yasuda, K. *Chem. Abstr.* **62**: P10491d (1965).
14. Mazac, R. and K. Syhora. Steroid derivatives. LXVII. Dependence of alcoholysis of 6-halo-4-pregna-3-ones on sterical factors. *Coll. Czech. Chem. Commun.* **3**: 1547–1557 (1970).
15. Teutsch, G., L. Weber, G. Page, E. L. Shapiro, H. L. Herzog, R. Neri and E. J. Collins. Influence of 6-azido and 6-thiocyanato substitution on progestational and cortical activities and a structure–activity correlation in the Δ^6-6-substituted progestational series. *J. Med. Chem.* **16**: 1370–1376 (1973).
16. Duax, W. L., V. Cody and J. Hazel. Steroid structure and function. I. Conformational transmission in 17α-acetoxy progesterone. *Steroids* **30**: 471–480 (1977).
17. Duax, W. L., V. Cody, J. Griffin, J. Hazel and C. M. Weeks. Steroid structure and function. II. Conformational transmission and receptor binding of medroxyprogesterone acetate. *J. Steroid Biochem.* **9**: 901–907 (1978).
18. Shapiro, E. L., T. L. Popper, L. Weber, R. Neri and H. L. Herzog. The synthesis and progestational activity of some 12α-cyclomethylene-16-methylene progesterone derivatives. *J. Med. Chem.* **12**: 631–636 (1969).
19. Duax, W. L. and D. A. Norton (eds.). *Atlas of Steroid Spectra, Vol. 1*. IFI/Plenum, New York (1975).
20. Duax, W. L. and P. D. Strong. Steroid structure and function. V. A-Ring conformation in 17α-hydroxy-6α-methylprogesterone. *Steroids* **34**: 501–508 (1979).
21. Fullerton, D. S., K. Yoshioka, D. C. Rohrer, A. H. L. From and K. Ahmed. Digitalis genin activity: Side-group carbonyl oxygen position is a major determinant. *Science* **205**: 917–919 (1979).

Send reprint requests to: Frank S. LaBella, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada, R3E 0W3.