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EFFECTS OF CHLORPROMAZINE AND IMIPRAMINE ON RAT HEART SUBCELLULAR MEMBRANES*

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Abstract—The effects of chlorpromazine and imipramine at concentrations ranging from 25 to 120 μM on ATPase activities, as well as calcium binding and uptake abilities of the rat heart subcellular membranes, were studied *in vitro*. Chlorpromazine significantly decreased calcium binding, Mg^{2+} ATPase and Na^+–K^+ ATPase activities of the sarcolemmal fraction, whereas imipramine decreased calcium binding, Ca^{2+} ATPase and Mg^{2+} ATPase activities. Chlorpromazine also produced significant inhibition of the calcium binding and uptake abilities of the microsomal and mitochondrial fractions, while imipramine depressed the mitochondrial calcium uptake activity only at concentrations of 80 μM or higher. The mitochondrial respiratory and oxidative phosphorylation activities were depressed at high concentrations of these drugs. Since different membrane systems have been considered to be involved in the regulation of heart function and metabolism, the observed decreases in ATPase and calcium-accumulating activities of the heart subcellular membranes may represent one of the molecular mechanisms for the cardiodepressant actions of chlorpromazine and imipramine.

Drugs such as chlorpromazine and imipramine, which are useful in the therapy of psychiatric patients, have also been demonstrated to produce negative chronotropic and inotropic effects on the intact dog heart [1], the isolated perfused rat heart [2], and the isolated rat atria [3]. Although antipsychotic agents have been reported to decrease calcium uptake, oxidative phosphorylation, and ATPase activities [4–6], no conclusion with respect to the molecular basis of action of these agents on myocardium can be drawn because these studies were carried out with liver and brain tissues. Since subcellular membranes have been considered to play an important role in the regulation of heart function and since alterations in their activities are believed to be involved in the development of myocardial contractile failure [7, 8], the present experiments were undertaken to examine the actions of chlorpromazine and imipramine on heart sarcolemma, sarcoplasmic reticulum (microsome) and mitochondria. We have employed 25–120 μM concentrations of both of these agents to study their effects on the biochemical activities of these subcellular membranes because depression of myocardial contractility by these agents has been observed at concentrations of 10 μM or higher [1, 9, 10].

METHODS

Male albino rats weighing 300–400 g were decapitated. The hearts were quickly removed and immersed in a cold buffer. The heart sarcolemmal fraction was isolated by the hypotonic shock–LiBr treatment method described elsewhere [11, 12]. The microsomal and mitochondrial fractions were isolated by differential centrifugation as described previously [13]. These fractions were purified and characterized in terms of marker enzyme activities and electron microscopic examination. As reported earlier [12], only minimal cross contamination was observed in the subcellular fractions employed in this study. The sarcolemmal Mg^{2+} ATPase and Ca^{2+} ATPase activities were measured at 37° in a medium containing 50 mM Tris–HCl (pH 7.4), 4 mM Tris–ATP, 4 mM $MgCl_2$ or 4 mM $CaCl_2$. The sarcolemmal total ATPase (Na^+–K^+ ATPase + Mg^{2+} ATPase) activity was measured in a medium containing 50 mM Tris–HCl (pH 7.4), 4 mM Tris–ATP, 4 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl and 1 mM EDTA. The Na^+–K^+ ATPase activity was calculated by subtracting the Mg^{2+} ATPase activity from the total ATPase activity. All the assay conditions employed here were for the optimal ATPase activities. The measurement of calcium binding activity by the sarcolemmal membrane was carried out in a medium containing 100 mM Tris–HCl (pH 7.4) and 1.25 mM $^{45}CaCl_2$ at 37°. The calcium binding activity represents ATP-independent binding; this sarcolemmal preparation has been shown to possess neither ATP-dependent calcium binding nor Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activities [14]. The details of the measurements of the sarcolemmal ATPase and calcium binding activities have been reported previously [11, 14–16].

Total ATPase activities of the microsomal and mitochondrial fractions were determined in a medium containing 20 mM Tris–HCl (pH 7.0), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mM $CaCl_2$ and 4 mM Tris–ATP in the presence or absence of 4 mM potas-

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sium oxalate, respectively [13]. The P_i liberated was measured by the method of Taussky and Shorr [17]. Treatment with activated charcoal was necessary to remove the drug which interfered during color development in the determination of P_i [18]. Energy-dependent calcium binding and uptake activities of both the mitochondrial and microsomal fractions were studied by the millipore filtration technique [13]. The incubation medium for calcium binding activities (25°) contained 100 mM KCl, 10 mM $MgCl_2$, 0.1 mM $^{45}CaCl_2$, 4 mM ATP and 20 mM Tris–HCl (pH 7.0), whereas that for microsomal calcium uptake activity (37°) also contained 4 mM potassium oxalate. The mitochondrial calcium uptake activity (37°) was measured in the same medium as for calcium binding except that 4 mM KH_2PO_4 and 4 mM succinate were also present. The details of the experimental conditions, as well as of the measurements of calcium binding and uptake activities, have been described earlier [13], and it is understood that calcium binding and uptake imply, arbitrarily, calcium accumulation in the absence and presence of permeant ions respectively. The oxidative phosphorylation activity of the mitochrondrial fraction was determined according to the polarographic method of Sordahl *et al.* [19], by using a Clark oxygen electrode and a Gilson oxygraph. The assay medium contained 250 mM sucrose, 10 mM K_2HPO_4, 1.5 mM pyruvate, 0.3 mM malate and 10 mM Tris–HCl (pH 7.4). State 3 respiration was initiated by the addition of 250 nmoles ADP, whereas state 4 refers to the condition when all ADP in the medium has been phosphorylated. The respiratory control index (RCI) was calculated as the ratio of oxygen consumption during states 3 and 4, whereas the phosphorylation rate was calculated by multiplying the values for ADP:O ratio and state 3 respiration. Protein concentration was estimated by the method of Lowry *et al.* [20]. The results were analyzed statistically by the paired *t*-test. In some cases analysis of variance was also used for testing the statistical significance; the conclusions derived were the same as those with the paired *t*-test.

RESULTS

In one set of experiments, effects of various concentrations of chlorpromazine and imipramine on heart sarcolemmal calcium binding and ATPase activities were studies, and the results are shown in Table 1. Both of these agents inhibited ($P < 0.05$) dose dependently the calcium binding activity at concentrations of 50–120 μM. The Ca^{2+} ATPase activity was not affected by chlorpromazine but was significantly decreased by high concentrations of imipramine (120 μM). Similar results were obtained when 1.25 mM Ca^{2+} was used in the assay medium for determining Ca^{2+} ATPase activity (control value under these conditions was 21.5 μmoles P_i/mg of protein/hr). The Mg^{2+} ATPase was depressed by these agents at concentrations of 80 and 120 μM. It is noteworthy that the Na^+–K^+ ATPase activity was significantly inhibited by all concentrations of chlorpromazine employed (25–120 μM), but not by imipramine. Although chlorpromazine at a 10 μM concentration inhibited the Na^+–K^+ ATPase activity by 10–15 per cent, this action was not statistically significant.

In another set of experiments, effects of chlorpromazine and imipramine on heart microsomal calcium-accumulating ability and Ca^{2+} + Mg^{2+} (total) ATPase activity were investigated, and the results are given in Table 2. Imipramine was found to have no significant effects on the calcium-accumulating and total ATPase activities of the microsomal fraction. On the other hand, chlorpromazine significantly decreased microsomal calcium binding and total ATPase activities at concentrations from 80 to 120 μM and calcium uptake activity at concentrations from 50 to 120 μM. The effects of chlorpromazine on microsomal calcium uptake activity were also tested by employing different concentrations of calcium in the incubation medium. The control calcium uptake activities at 1, 5, 10, 25 and 50 μM concentrations of calcium were 9 ± 2, 60 ± 9, 84 ± 13, 110 ± 16 and 140 ± 21 nmoles Ca^{2+}/mg of protein/5 min (mean ± S.E. of three experiments)

Table 1. Effects of imipramine and chlorpromazine on rat heart sarcolemmal calcium binding, Ca^{2+} ATPase, Mg^{2+} ATPase and Na^+–K^+ ATPase activities*

Concn (μM)	Calcium binding (nmoles Ca^{2+}/mg protein/5 min)	ATPase activities (μmoles P_i/mg protein/hr)		
		Ca^{2+} ATPase	Mg^{2+} ATPase	Na^+–K^+ ATPase
Control	204 ± 26	32.7 ± 2.8	22.1 ± 1.7	9.7 ± 0.6
Chlorpromazine				
25	185 ± 19	31.4 ± 3.1	21.4 ± 1.7	6.3 ± 0.8†
50	146 ± 18†	30.2 ± 2.6	18.9 ± 1.8	5.5 ± 0.7†
80	137 ± 15†	29.4 ± 2.7	14.6 ± 1.4†	3.4 ± 0.5†
120	126 ± 9†	27.5 ± 2.6	12.2 ± 1.1†	1.6 ± 0.3†
Imipramine				
25	166 ± 18	31.6 ± 2.5	22.5 ± 1.9	9.5 ± 0.7
50	100 ± 9†	30.4 ± 2.7	21.2 ± 1.8	9.1 ± 0.6
80	87 ± 11†	28.5 ± 2.8	17.4 ± 1.3†	8.8 ± 0.7
120	81 ± 8†	25.3 ± 1.9†	14.6 ± 1.2†	8.6 ± 0.8

* Each value is the mean ± S.E.M. of four experiments.
† Significantly different from the control, $P < 0.05$.

Table 2. Effects of imipramine and chlorpromazine on rat heart microsomal calcium binding, calcium uptake and total ATPase activities*

Concn (μM)	Calcium accumulation (nmoles Ca^{2+}/mg protein/5 min)		ATPase activity (μmoles P_i/mg protein/min)
	Calcium binding	Calcium uptake	
Control	31.3 ± 2.9	155 ± 16	2.18 ± 0.29
Chlorpromazine			
25	29.1 ± 2.5	127 ± 14	2.03 ± 0.31
50	26.0 ± 2.4	68 ± 9†	1.96 ± 0.25
80	21.5 ± 2.6†	58 ± 11†	1.76 ± 0.14†
120	18.3 ± 1.9†	36 ± 6†	1.51 ± 0.16†
Imipramine			
25	32.8 ± 2.7	155 ± 21	2.28 ± 0.27
50	32.7 ± 2.6	160 ± 18	2.15 ± 0.25
80	32.3 ± 2.8	145 ± 15	2.15 ± 0.31
120	28.8 ± 2.8	149 ± 17	2.05 ± 0.27

* Each value is the mean ± S.E.M. of four experiments.
† Significantly different from the control, $P < 0.05$.

Table 3. Effects of imipramine and chlorpromazine on rat heart mitochondrial calcium binding, calcium uptake and total ATPase activities*

Concn (μM)	Calcium accumulation (nmoles Ca^{2+}/mg protein/5 min)		ATPase activity (μmoles P_i/mg protein/min)
	Calcium binding	Calcium uptake	
Control	41.2 ± 4.1	98.0 ± 8.5	0.94 ± 0.11
Chlorpromazine			
25	38.9 ± 3.6	82.5 ± 6.5†	0.91 ± 0.07
50	37.2 ± 3.5	74.1 ± 6.8†	0.87 ± 0.10
80	29.8 ± 2.6†	55.4 ± 5.4†	0.82 ± 0.09
120	20.9 ± 2.5†	44.3 ± 3.1†	0.80 ± 0.12
Imipramine			
25	39.1 ± 4.6	96.9 ± 8.7	0.94 ± 0.08
50	37.8 ± 3.9	88.8 ± 8.9	0.88 ± 0.09
80	34.4 ± 4.1	80.0 ± 6.5†	0.85 ± 0.09
120	34.4 ± 3.8	68.1 ± 6.9†	0.81 ± 0.10

* Each value is the mean ± S.E.M. of four experiments.
† Significantly different from the control, $P < 0.05$.

Table 4. Effects of imipramine and chlorpromazine on rat heart mitochondrial oxidative phosphorylation activity*

Concn (μM)	Oxygen consumption (natoms O/mg protein/min)		RCI	ADP:O	Phosphorylation rate (nmoles ADP/mg protein/min)
	State 3	State 4			
Control	125 ± 5	16.0 ± 1.8	7.9 ± 0.7	2.7 ± 0.2	332 ± 13
Chlorpromazine					
25	127 ± 4	18.6 ± 1.4	6.8 ± 0.6	2.7 ± 0.3	340 ± 14
50	126 ± 5	21.0 ± 1.0†	5.9 ± 0.4†	2.5 ± 0.2	316 ± 18
80	128 ± 5	25.2 ± 0.8†	5.1 ± 0.5†	2.4 ± 0.2	308 ± 14
120	108 ± 5†	29.0 ± 1.2†	3.7 ± 0.4†	2.0 ± 0.2†	214 ± 10†
Imipramine					
25	122 ± 4	17.0 ± 1.2	7.3 ± 0.5	2.7 ± 0.3	330 ± 14
50	122 ± 5	19.0 ± 1.0	6.4 ± 0.6	2.6 ± 0.2	314 ± 12
80	127 ± 5	21.2 ± 1.2†	5.9 ± 0.5†	2.5 ± 0.1	316 ± 12
120	123 ± 4	25.8 ± 1.4†	4.7 ± 0.3†	2.3 ± 0.1†	288 ± 11†

* Each value is the mean ± S.E.M. of four experiments.
† Significantly different from the control, $P < 0.05$.

respectively. These activities were depressed 50–60 per cent by 50 μM chlorpromazine. In three experiments, Ca^{2+}-stimulated Mg^{2+}-dependent ATPase activity in the microsomal fraction was also measured according to a method described earlier [12]. Microsomal Ca^{2+}-stimulated Mg^{2+}-dependent ATPase activities in the absence and presence of 50 μM chlorpromazine were 0.32 ± 0.04 and 0.13 ± 0.02 μmole P_i/mg of protein/min, respectively.

Actions of chlorpromazine and imipramine on mitochondrial calcium binding, calcium uptake and total ATPase activities were measured, and the results are given in Table 3. Chlorpromazine significantly depressed calcium binding activity at concentrations of 80–120 μM. Significant inhibition of calcium uptake activity by chlorpromazine was seen at all concentrations employed in the present study (25–120 μM). On the other hand, imipramine did not depress calcium binding and total ATPase activities significantly. The calcium uptake ability of mitochondria was, however, significantly depressed by 80–120 μM imipramine. As reported previously [12], the mitochondrial fraction, unlike the microsomal fraction, did not show Ca^{2+}-stimulated Mg^{2+}-dependent ATPase activity. In other experiments, effects of chlorpromazine and imipramine on the mitochondrial oxidative phosphorylation activity were examined, and the data are shown in Table 4. Both chloropromazine and imipramine significantly decreased the RCI at concentrations of 50–120 μM and 80–120 μM respectively. At a concentration of 120 μM, both drugs significantly depressed the ADP:O ratio and the phosphorylation rate of the mitochondrial fraction.

DISCUSSION

In this study we have shown that imipramine and chlorpromazine (50–120 μM) decreased sarcolemmal calcium binding and Mg^{2+} ATPase activities. Furthermore, sarcolemmal Na^+–K^+ ATPase activity was depressed by chlorpromazine (25–120 μM), whereas sarcolemmal Ca^{2+} ATPase activity was decreased by high concentrations of imipramine (120 μM). These observations suggest that both chlorpromazine and imipramine may act on heart sarcolemma. Chlorpromazine at a 1 mM concentration has also been reported to decrease the nonspecific calcium binding activity of the adrenal medullary plasma membrane [21]. Although imipramine in concentrations of 25–120 μM failed to decrease the sarcolemmal Na^+–K^+ ATPase activity significantly, a depressant effect of this agent at high concentrations cannot be ruled out. This view is consistent with the feelings of Verrill *et al.* [22], who have shown significant inhibition of rat heart sarcolemmal Na^+–K^+ ATPase activity by imipramine at concentrations of 600 μM or higher. Furthermore, 50 per cent inhibition of rat brain Na–K^+ ATPase activity by imipramine and chlorpromazine was seen at 1.5 and 0.15 mM, respectively [23]. At any rate, it can be conceived that alterations in calcium binding and ATPase activities of heart sarcolemma by both chlorpromazine and imipramine may produce changes in ion fluxes across the cell membrane [7, 8], and these changes may explain, in part, the negative inotropic action of these agents. Similar changes in the sarcolemmal calcium binding and ATPase activities have been reported to occur in the presence of other cardiodepressant agents such as propranolol, quinidine and divalent cations [11, 15, 16], as well as under several pathological situations [8].

Since chlorpromazine was also found to decrease microsomal calcium binding and calcium uptake activities, it is likley that the cardiodepressant effects of chlorpromazine are partly mediated by its action on the sarcoplasmic reticulum. Although the implication of this mechanism in decreasing cardiac contractile force has been outlined elsewhere [7, 8], it should be mentioned that several cardiodepressants, such as barbiturates [24, 25], ethanol [26], propranolol [27] and quinidine [18], have been shown to decrease calcium-accumulating abilities of heart microsomal and mitochondrial fractions. In this regard, imipramine seems to be different from chlorpromazine, since imipramine did not affect significantly the microsomal calcium accumulation at concentrations employed in this study. This is further supported by the fact that chlorpromazine, unlike imipramine, decreased the microsomal total ATPase activity, probably due to inhibition of the Ca^{2+}-stimulated ATPase, which has been shown to be linked with microsomal calcium transport [7, 8]. Our findings concerning the actions of these agents on heart microsomal calcium uptake and ATPase activities are consistent with those of other investigators who employed skeletal muscle microsomal preparations [28–30]. Cardiac microsomal Ca^{2+}-stimulated Mg^{2+}-dependent ATPase activity has also been reported to decrease due to 0.20–2.4 mM chlorpromazine [31].

Mitochondria, in addition to playing an important role in the generation of ATP through the process of oxidative phosphorylation, have also been reported to accumulate calcium by an energy-dependent mechanism [7, 8]. Although the exact contribution of mitochondria in the regulation of the intracellular concentration of calcium is not clear at present, it is interesting to note that chlorpromazine, in concentrations affecting microsomal calcium accumulation, also depressed mitochondrial calcium binding and uptake activities. Furthermore, chlorpromazine was effective in producing alterations in the mitochondrial respiratory and oxidative phosphorylation abilities. Although imipramine also produced changes in mitochondrial respiration, oxidative phosphorylation and calcium uptake similar to those seen with chlorpromazine, it should be noted that mitochondrial calcium binding as well as state 3 respiration were not affected significantly by imipramine at the concentrations employed in this study. Tjioe *et al.* [32] have also reported depressant effects of chlorpromazine and imipramine on rat brain mitochondrial calcium uptake. The observed changes in mitochondrial oxidative phosphorylation in the presence of chlorpromazine and imipramine were not associated with changes in mitochondrial ATPase activities, and this may suggest a complex mode of action of these agents on the mitochondrial membrane.

From the results presented in this study it can be seen that chlorpromazine and imipramine at 50 μM

concentrations inhibited sarcolemmal Ca^{2+} binding activity. Such a decrease in Ca^{2+} binding caused by these agents can be conceived to reduce sarcolemmal Ca^{2+} stores which are available for release upon depolarization of the myocardium. This would then account for the negative inotropic effect of chlorpromazine and imipramine if it is assumed that the sensitivity of the isolated sarcolemmal preparation to these agents under *in vitro* conditions is markedly less than that seen under *in vitro* situations. In this regard it should be noted that these agents have been shown to produce cardiodepressant effects at 10 μM concentrations [1, 9, 10]. Although sarcolemmal Na^+–K^+ ATPase activity was diminished at a 25 μM concentration of chlorpromazine, this action may not readily explain the negative inotropic effect of this agent because the positive inotropic action of cardiac glycosides has been considered to be associated with an inhibition of this enzyme activity [33]. In this regard, however, Schwartz *et al.* [33] have suggested that the binding of cardiac glycosides to Na^+–K^+ ATPase produces a positive inotropic effect by forcing the enzyme to take a particular conformation, which is essential for an increased calcium influx associated with membrane depolarization. Thus, it may be proposed that chlorpromazine inhibits Na^+–K^+ ATPase by locking the enzyme in another conformation and hence decreasing the force of contraction. Although other sarcolemmal ATPase activities were also inhibited by these agents, the concentrations required for this action were too high to draw any meaningful conclusion. Likewise, the participation of mitochondrial and microsomal sites in the negative inotropic action of these agents cannot be readily accepted because these membranes were affected by concentrations of 25 μM or higher. However, decreased sensitivity to drugs of these membrane fractions upon isolation and purification, as well as the absence of several cytoplasmic factors under *in vitro* conditions, cannot be ignored. Furthermore, it has to be assumed that these agents are capable of entering the myocardial cell in concentrations sufficient to influence these sites. In spite of these general problems in extrapolating *in vitro* data to *in vivo* situations, the results described in this study indicate that the cardiodepressant effects of high concentrations of chlorpromazine and imipramine may be associated with their actions on the mitochondrial, sarcoplasmic reticulum and sarcolemmal membrane systems.

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