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Potassium channel dysfunction in fibroblasts identifies patients with Alzheimer disease

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ABSTRACT **Since memory loss is characteristic of Alzheimer disease (AD), and since K^+ channels change during acquisition of memory in both molluscs and mammals, we investigated K^+ channel function as a possible site of AD pathology and, therefore, as a possible diagnostic index as well. A 113-pS tetraethylammonium (TEA)-sensitive K^+ channel was consistently absent from AD fibroblasts, while it was often present in young and aged control fibroblasts. A second (166-pS) K^+ channel was present in all three groups. Elevated external potassium raised intracellular Ca^{2+} in all cases. TEA depolarized and caused intracellular Ca^{2+} elevation in young and aged control fibroblasts but not AD fibroblasts. The invariable absence of a 113-pS TEA-sensitive K^+ channel and TEA-induced Ca^{2+} signal indicate K^+ channel dysfunction in AD fibroblasts. These results suggest the possibility of a laboratory method that would diagnostically distinguish AD patients, with or without a family history of AD, from normal age-matched controls and also from patients with non-AD neurological and psychiatric disorders.**

Clinical diagnosis of Alzheimer disease (AD) includes satisfaction of several criteria such as prolonged loss of memory, impaired social and/or work functions, gradual onset, and progressive decline. A clinical diagnosis of AD also requires exclusion of a host of other neurological, psychiatric, and medical disorders (1). Even in the absence of these other disorders, however, AD patients may not satisfy enough clinical criteria to be clearly distinguished from other individuals who are simply showing the signs of old age. Thus, all-too-common errors in antemortem diagnosis of AD (2) might be significantly reduced by a laboratory test that does distinguish AD from old age and from other neurological and psychiatric disorders.

Designs for such a laboratory test are suggested by what is known at present about AD etiology and pathophysiology. AD has been directly and indirectly linked to a genetic origin (3–5). Proposed causes and/or predisposing factors have ranged from defects in β-amyloid protein metabolism (6–12) and environmental factors (13, 14) to abnormal Ca^{2+} homeostasis and/or Ca^{2+}-activated kinases (15–22). Since memory loss is characteristic of AD (14) and since K^+ channels change during acquisition of memory in both molluscs and mammals (23–27), we investigated K^+ channel function as a possible site of AD pathology and, therefore, as a possible diagnostic index as well. If AD is a systemic process involving multiple organ systems, derangement of K^+ channel function might occur within membranes of diverse cell types and not only in neurons during the clinical onset of the disease. Indeed, using patch-clamp (28, 29) and calcium-imaging techniques (30, 31), we report here evidence that a 113-pS K^+ channel is functionally absent in fibroblasts of AD patients but not in fibroblasts of age-matched control (AC) or young control (YC) individuals.

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METHODS

Cell Lines. Cultured skin fibroblasts from the Coriell Cell Repositories (Camden, NJ) were grown under highly standardized conditions (32). Six YC (three males, three females; 21.5 ± 2.8 years, mean ± SD), five AC (one male, four females; 65.2 ± 6.0 years), and seven AD (three males, four females; 60.5 ± 6.8 years) cell lines were used for patch-clamp experiments. Five AD lines were from familial patients. Some of the lines (two AC and four AD) were from the Canadian kindred (33) (Table 3). Additional description of these cell lines can be found elsewhere (34, 35). In agreement with the literature, our data indicate the time to senescence and growth rate do not vary between the AD and control lines (YC and AC) (36). Cells were seeded (≈5 cells per mm^2) in 35-mm Nunc Petri dishes [in Dulbecco's modified Eagle's medium (DMEM; GIBCO), supplemented with 10% fetal calf serum] and used when cell density was equivalent for all cell lines (50 cells per mm^2).

Electrophysiology and Single-Channel Analysis. Patch-clamp experiments were performed at room temperature (21–23°C), following standard procedures (28). Membrane potential was measured both prior to obtaining outside-out patches and by using the "perforated-patch" technique (28, 37). Before recording, culture medium was replaced with the following solution (mM): NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, Hepes (NaOH) 10, pH = 7.4. Pipettes were made from Blue Tip capillary tubes (i.d. 1.1–1.2 mm) by using a BB-CH Mecanex puller and then filled with a high-K^+ solution (mM): KCl 140, $CaCl_2$ 2, $MgCl_2$ 1, Hepes (NaOH) 10, pH = 7.4. Pipette resistances were ≈6 MΩ. Records were obtained by using an Axopatch-1C amplifier (dc to 10 kHz), stored on tape (Toshiba PCM video recorder), and later transferred to a personal computer using an Axolab interface. Only recordings lasting for at least 3 min were considered for final analysis. The pClamp suite of programs was used for single-channel data acquisition and analysis. Amplifier, interface, and software were obtained from Axon Instruments (Foster City, CA).

Ca^{2+} Imaging. Following the same cell-culture procedures used for patch-clamp experiments, we used 13 AD [60.5 ± 5.9 years (mean ± SD); 7 males, 6 females (5 from the Canadian kindred)], 10 AC [62.9 ± 4.6 years; 7 females, 3 males (1 from the Canadian kindred)], and 6 YC (21.5 ± 2.8 years; 3 males, 3 females) cell lines for the calcium-imaging experiments. Culture medium was replaced (three washes) with the following solution (mM): NaCl 140, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1.5, glucose 5, Hepes (NaOH) 10, pH = 7.4. Then, fura-2AM (the acetoxymethyl ester; Molecular Probes) was added to a final

Abbreviations: AD, Alzheimer disease; AC, age-matched control; YC, young control; TEA, tetraethylammonium.

concentration of 2 μM and cells were incubated at room temperature for 60 min. After incubation, cells were washed at least three times with the previously described solution before the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, was determined. Fluorescence was measured with a Hamamatsu Argus 50 imaging system (Hamamatsu Photonics, Japan) under the control of a personal computer (Hamamatsu imaging software package U4469). Excitation at 340 nm and 380 nm was attenuated with neutral density filters. Fluorescent images were obtained with a 400-nm dichroic mirror and a 510-nm long-pass barrier filter. The objective was a ×10 Nikon UV fluor. Actual fluorescence was measured within a uniformly illuminated fraction (¼) of the whole image. The Ca^{2+} images were expressed as the ratio between 510-nm fluorescent images illuminated by 340-nm light and those illuminated by 380-nm light. The ratio values were transformed to absolute $[Ca^{2+}]_i$ values after calibration (A. Miyakawa, personal communication) based on the following equation:

$$R = R_{max} + (R_{min} - R_{max})/[1 + ([Ca^{2+}]_i/K_d)^b],$$

in which R denotes F_{340}/F_{380}, and R_{max} and R_{min} are the values of R when the concentration of calcium is at a maximum and a minimum, respectively. K_d is a dissociation constant of fura-2 for Ca^{2+} and was determined as 240 nM. The value of b, which determines the degree of asymmetry, was 1.2. Tetraethylammonium (TEA) application caused a minimum of 100% $[Ca^{2+}]_i$ elevation in at least 18% of cells in every control cell line except one YC. A response of 100% $[Ca^{2+}]_i$ elevation in at least 10% of cells in a line was, therefore, considered to be conservative criterion for a positive response. Only one AD cell line had cells with any response (100% $[Ca^{2+}]_i$ elevation in 4%), well below the criterion.

RESULTS

Electrophysiology. On average, fibroblasts from AD patients and controls took essentially the same time [YC = 3.6 ± 0.2 days (n = 6); AC = 3.4 ± 0.1 days (n = 10); AD = 3.5 ± 0.1 days (n = 13) (mean ± SEM)] to reach criterion density (50 cells per mm²) from the same initial seeding density (5 cells per mm²). Thus, selection of cells with different channel properties because of AD-specific differences in growth rates was very unlikely, if not impossible. In the cell-attached mode, two types of K^+ channels were recorded from human skin fibroblasts. Since pipettes were filled with a high-K^+ solution, K^+ currents were inward as expected, and their reversal potential approximately corresponded to the cell resting potential (Fig. 1). Both channels had linear voltage–current relationships, with slope conductances of 113 and 166 pS, respectively (Fig. 1). At 0-mV pipette potential, the channels could easily be identified by their unitary current size (Fig. 1 *A* and *C*) and by their percentages of open time, ≈60% for the 113-pS K^+ channel and ≈10% for the 166-pS K^+ channel. For both channels, the percentages of open time showed no significant voltage dependence (+60 to −40 mV pipette potential). The 113-pS channel was present in 47% of the YC cells (n = 30) and in 94% of the AC cells (n = 17), while it was never found in fibroblasts from AD patients (n = 24) (Table 1). There were no AD cell lines (n = 7) that had fibroblasts with an observable 113-pS channel. By contrast, all AC cell lines (n = 5) and three of six YC cell lines had fibroblasts with observable 113-pS channels. The 166-pS channel was found with similar frequencies in all three groups (Table 1). Using cell-free patches, we observed that both

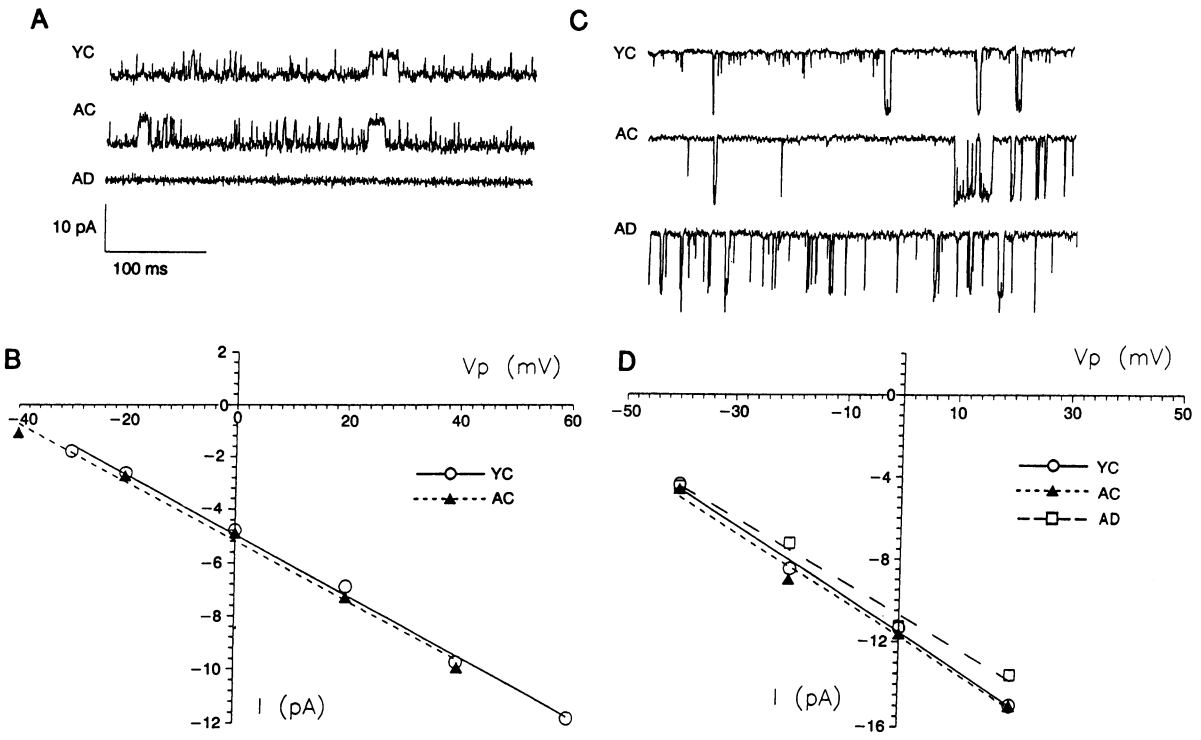

FIG. 1. Cell-attached recordings from AD and control fibroblasts. K^+ channels of ≈4.5-pA unitary current size (0-mV pipette potential) with identical kinetics appeared in YC and AC fibroblasts, but such a channel was entirely absent from the recording of AD fibroblasts (*A*). Downward deflections represent the open state. *I/V* relationships (*B*) and slope conductances (determined by linear regression) were almost identical within the voltage range explored, 113.2 ± 0.9 pS (mean ± SD, n = 8) for YC fibroblasts and 112.9 ± 3.2 pS (n = 7) for AC fibroblasts. A second channel (166 pS) was recorded under the same conditions from fibroblasts of all three groups (*C*). *I/V* relations (*D*) as well as conductances (YC = 173.4 ± 5.7 pS, n = 4; AC = 169.2 ± 2.8 pS, n = 4; AD = 157.6 ± 4.7 pS, n = 6) were approximately the same across groups. Membrane potentials were similar in control (−42.6 ± 5.4 mV, n = 7) and AD (−45.4 ± 6.9 mV, n = 3) fibroblasts.

Table 1. Distribution of the two K^+ channels in YC, AC, and AD fibroblasts

Fibroblasts	No. of cell lines (cells) Total	113-pS K^+ channel	166-pS K^+ channel
YC	6 (30)	3 (14)	4 (6)
AC	5 (17)	5 (16)	3 (6)
AD	7 (24)	0 (0)	4 (4)

The 113-pS channel was found with significantly higher frequency in YC (47%) and AC (94%), compared with AD (0%) fibroblasts; $\chi^2 = 18.96$, $P < 0.001$. The 166-pS channel was present in fibroblasts from every group, with about the same frequency ($\chi^2 = 0.89$, not significantly different). None of the seven AD cell lines examined had fibroblasts with an observable 113-pS channel, while it was present in three YC cell lines and in five AC cell lines; $\chi^2 = 11.93$, $P < 0.005$.

channels were sensitive to 50 mM Ba^{2+} (inside-out, $n = 4$ for each channel), but only the 113-pS channel was sensitive (outside-out, $n = 4$ YC, $n = 3$ AC) to the K^+ channel blocker TEA (Ba and TEA were added as chlorides). The TEA blockade of the 113-pS channels (possibly together with other channels) significantly affects membrane potential, since control cells ($n = 4$) depolarized 13–20 mV after 100 mM TEA addition.

Ca^{2+} Imaging. Depolarization of the fibroblasts by perfusion in elevated external K^+ caused greater elevation of $[Ca^{2+}]_i$ as measured by fura-2 fluorescence imaging (30, 31) in YC as compared with AC and AD cells, which were not significantly different from each other. This depolarization-induced $[Ca^{2+}]_i$ increase was eliminated by decreasing external Ca^{2+} or by adding Ca^{2+} channel blockers (Fig. 2), and thus it arose from Ca^{2+} influx through voltage-dependent channels. As expected, depolarization of control (YC and AC) fibroblasts by TEA also caused $[Ca^{2+}]_i$ elevation (Fig. 3, Table 2), which was eliminated by lowering external Ca^{2+} or by adding Ca^{2+} channel blockers. AD fibroblasts, however, showed $[Ca^{2+}]_i$ elevation only in increased external K^+ and had no $[Ca^{2+}]_i$ response that met criterion with addition of even 100 mM TEA (Table 2, Fig. 3). Every AC cell line ($n = 10$) and all but one YC cell line ($n = 6$) had cells with criterion responses to 100 mM TEA, while none of the 13 AD cell lines examined had cells with criterion responses (Table 2). Measurements of TEA-induced $[Ca^{2+}]_i$ elevations were repeated with a coded subsample that included AD and control fibroblasts. Experiments and analyses were conducted without the experimenter's knowledge of the cell lines' identities. The results were in complete agreement with the nonblind sample. None of the blindly examined AD cell lines ($n = 11$) showed $[Ca^{2+}]_i$ elevation in response to TEA, and all but one of the control cell lines (4 AC and 6 YC) had TEA responses (Table 3). Since $[Ca^{2+}]_i$ elevation in response to high external K^+ concentration was virtually the same for AC and AD cells, the lack of AD cells' response to TEA is almost certainly due to dysfunction of K^+ channels and not to dysfunction of Ca^{2+} channels.

Table 3 summarizes all of the measurements made and all of the individuals included in this study. It is worth noting that three AD patients had autopsy-confirmed diagnoses. Five patients were from the same family, four being distant relatives, and two other patients were from another family.

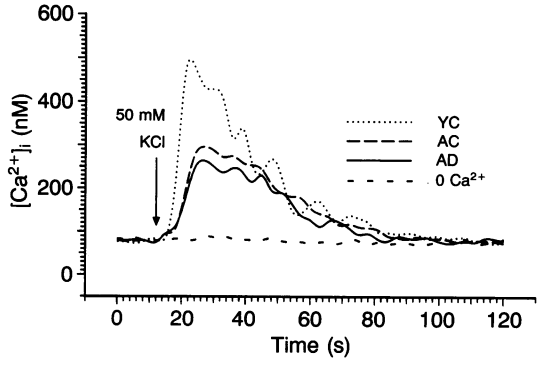

FIG. 2. Potassium-induced $[Ca^{2+}]_i$ elevation. Sample traces of time courses of the Ca^{2+} responses after addition of 50 mM KCl. The $[Ca^{2+}]_i$ peak occurs 10–15 s after stimulation, and $[Ca^{2+}]_i$ returns to basal levels after 100 s. No responses were observed when external Ca^{2+} concentration was lowered ["nominally Ca^{2+}-free" solution or 5 mM EGTA addition (estimated free $Ca^{2+} = 0.04$ μM)] or Ca^{2+} channel blockers (0.1 mM $LaCl_3$, 10 mM $CoCl_2$, 10 mM $NiCl_2$, 10 mM $CdCl_2$, or 10 μM nifedipine) were added before stimulation ("0 Ca^{2+}"). High K^+-induced depolarization caused $[Ca^{2+}]_i$ elevation (at least 100% increase) in all three groups (AD $n = 13$ cell lines; AC $n = 10$; YC $n = 6$).

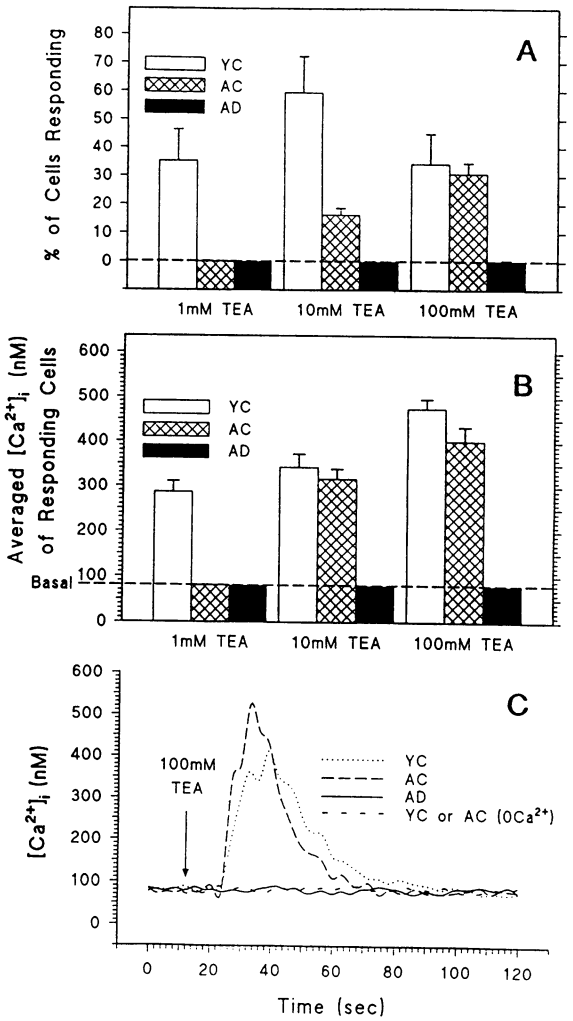

FIG. 3. $[Ca^{2+}]_i$ elevation in response to TEA. (*A*) Application of 1 mM TEA increased $[Ca^{2+}]_i$ in YC fibroblasts ($n = 130$ cells) but not in AC fibroblasts ($n = 184$) or AD fibroblasts ($n = 195$). TEA at 10 mM increased $[Ca^{2+}]_i$ in YC ($n = 176$), AC ($n = 231$), but not AD ($n = 204$) fibroblasts ($\chi^2 = 134.00$, $P << 0.001$). Similarly, 100 mM TEA increased $[Ca^{2+}]_i$ in YC ($n = 532$) and AC ($n = 417$) but not AD ($n = 738$) fibroblasts ($\chi^2 = 231.44$, $P << 0.001$) (also see Table 2). (*B*) $[Ca^{2+}]_i$. Basal $[Ca^{2+}]_i$ levels were virtually the same (SEM < 0.5 nM), therefore standard error bars are not distinguishable from the bar representing the arithmetic mean for those groups. (*C*) Time courses of the Ca^{2+} responses. The $[Ca^{2+}]_i$ peak occurs 20–30 s after 100 mM TEA addition in YC and AC fibroblasts, and $[Ca^{2+}]_i$ returns to basal levels after 100 s. Note that no response meeting criterion (10% of cells in a line with ≥100% elevation) was observed in AD cells. Similarly, there was no response in control cells when external Ca^{2+} concentration was lowered.

Table 2. Effect of 100 mM TEA on $[Ca^{2+}]_i$ in YC, AC, and AD fibroblasts

Fibroblasts	No. of cell lines (cells)	
	Total	TEA-induced $[Ca^{2+}]_i$ increase
YC	6 (532)	5 (145)
AC	10 (417)	10 (119)
AD	13 (738)	0 (4)

TEA caused $[Ca^{2+}]_i$ elevation in 27% of the YC fibroblasts and in 29% of the AC cells. By contrast, only 4 of the 738 AD fibroblasts examined responded to TEA ($\chi^2 = 231.44$, $P << 0.001$). All AC ($n = 10$) and five of six YC cell lines had cells responding to TEA, and none ($\chi^2 = 25.66$, $P < 0.001$) of the AD cell lines ($n = 13$) showed a TEA criterion response (i.e., 10% in a line ≥100% elevation).

There were, in addition, patients from six other families with members whose fibroblast K^+ channels were dysfunctional. All of the five AD lines from one family showed K^+ channel dysfunction, while both of the two AC lines from the same family showed normal K^+ channel function. The TEA-induced $[Ca^{2+}]_i$ elevation was also measured in a third control group, consisting of fibroblasts from neurological (Parkinson disease, Huntington disease, and Wernicke–Korsakoff syndrome) and psychiatric (schizophrenia) patients. Criterion (normal) TEA responses were observed in fibroblasts from the Parkinson patient, from the two schizophrenia patients, from all four Wernicke–Korsakoff patients, and from five of seven Huntington patients (Table 4). The TEA responses from this group are virtually identical to the responses found in YC and AC fibroblasts, while they are significantly different from the negative TEA response observed in AD fibroblasts ($P < 0.0001$, Fisher's exact test).

DISCUSSION

It should be pointed out that the patch-clamp method, because of its low success rate and because it samples only an extremely small fraction of the total cell membrane, has a much greater likelihood of missing K^+ channels than does the method of imaging TEA-elicited Ca^{2+} signals. This was why two independent measures of K^+ channel function were used here. Nevertheless, the patch-clamp and $[Ca^{2+}]_i$ measurements were in good agreement insofar as they both indicated K^+ channel dysfunction in the AD fibroblasts. The 113-pS channel that was found by the patch-clamp to be absent from AD fibroblasts could, of course, be "present" but not functional in AD. Such dysfunction could involve structural changes in the channel itself and/or alteration in processes involved in channel activity regulation. It will be important to

Table 3. Summary of measurements with AD, AC, and YC fibroblasts

Fibroblasts	Line	Age, yr	Sex	Race	Diagnostic criteria	113-pS K^+ channel	TEA response	
							Nonblind	Blind
AD	AG06840*	56	M	W	Clinical, family history	−	−	−
	AG06848*	55	F	W	Clinical, family history, autopsy	−	−	NT
	AG07637*	55	F	W	Clinical, family history	−	−	−
	AG08170*	56	M	W	Clinical, family history	−	−	−
	AG06844*	59	M	W	Clinical, family history, autopsy	NT	NT	−
	AG04400†	61	F	W	Clinical, family history	NT	NT	−
	AG04401†	53	F	W	Clinical, family history, autopsy	NT	−	−
	AG05809	63	F	W	Clinical, family history	−	−	NT
	AG08243	72	M	W	Clinical, no family history	−	−	−
	AG07375	71	M	W	Clinical, no family history	NT	−	−
	AG07376	59	M	W	Clinical, no family history	NT	−	−
	AG06263	67	F	W	Clinical, no family history	−	−	−
	AG07377	59	M	W	Clinical, no family history	NT	NT	−
AC	GM03524	67	F	B	Normal	+	+	NT
	AG06010	62	F	W	Normal	+	+	+
	AG06842*	75	M	W	Normal, family history	+	NT	NT
	AG07603*	61	F	W	Normal, family history	+	+	NT
	AG09878	61	F	B	Normal	+	+	+
	AG08044	58	F	B	Normal	NT	+	NT
	AG06241	61	M	W	Normal	NT	+	NT
	AG04560	59	M	W	Normal	NT	+	NT
	GM04260	60	M	W	Normal	NT	+	NT
	AG07141	66	F	W	Normal	NT	NT	+
	AG11363	74	F	W	Normal	NT	NT	+
YC	GM03652	24	M	W	Normal	+	+	+
	GM03651	25	F	W	Normal	+	+	+
	GM02987	19	M	W	Normal	−	−	−
	GM04390	23	F	W	Normal	+	+	+
	GM03377	19	M	W	Normal	−	+	+
	GM08399	19	F	?	Normal	−	+	+

AD fibroblasts were from familial ($n = 8$) and nonfamilial ($n = 5$) cases. Related individuals are indicated by footnotes. In all cases clinical diagnosis was made according to the *Diagnostic and Statistical Manual of Mental Disorders* (ref. 38, edition III or IIIR). Autopsy confirmed AD in three cases. Two of the AC ($n = 11$) cell lines are from unaffected members of the Canadian family 964. All YC lines ($n = 6$) are from normal individuals and individuals without AD family history. The presence of the 113-pS K^+ channel is indicated by +. Criterion $[Ca^{2+}]_i$ responses (to 100 mM TEA), indicated as +, were observed in all AC lines used and in all but one of the YC lines. None of the AD lines exhibited a response. A blind protocol was conducted to measure TEA responses in AD ($n = 11$) and control (YC = 6, AC = 4) fibroblasts. The results exactly reproduced those of the nonblind sample: no AD cell line exhibited TEA responses and 9 of 10 AC cells examined showed TEA responses ($\chi^2 = 17.33$, $P < 0.001$). W, white; B, black; NT, not tested.

*Members of the Canadian family 964; only AG06840 and AG06848 are immediate relatives (sibs).

†Members (sibs) of family 747.

Table 4. TEA-induced $[Ca^{2+}]_i$ responses in psychiatric and neuropathological non-AD fibroblasts

Line	Age, yr	Sex	Race	Diagnosis	TEA response
AG08395	85	F	W	Parkinson*	+
GM01835	27	F	W	Schizophrenia	+
GM02038	22	M	W	Schizophrenia	+
GM06274	56	F	W	Huntington	+
GM02165	55	M	W	Huntington	+
GM00305	56	F	W	Huntington	−
GM01085	44	M	W	Huntington	+
GM01061	51	M	W	Huntington	+
GM05030	56	M	W	Huntington	−
GM04777	53	M	W	Huntington	+
7504	50	M	W	Wernicke–Korsakoff	+
7505	52	F	W	Wernicke–Korsakoff	+
7507	63	M	W	Wernicke–Korsakoff	+
7508	64	M	W	Wernicke–Korsakoff	+

Fibroblasts from a Parkinson disease donor had normal TEA responses (indicated as +), which did not significantly differ from responses observed in the AC group. Fibroblasts from two schizophrenic patients also had normal TEA responses. In addition, normal TEA responses were observed in fibroblasts from five of seven Huntington disease patients. Fibroblasts from Wernicke–Korsakoff patients ($n = 4$) had normal TEA responses. The TEA responses are significantly different from those of AD fibroblasts to the level of $P < 0.0001$ (Fisher's exact test).
*Autopsy confirmation.

examine possible relationships of the changes reported here to derangement of β-amyloid protein metabolism (6–12) as well as previously implicated molecular mechanisms of associative memory (24). A systematic between-group variation of β-amyloid protein among fibroblasts has, thus far, never been reported, to our knowledge. Future research must concern such questions as well as the possible relevance of the present fibroblast findings for neurons and the functional integrity of the brain.

Since the TEA-elicited Ca^{2+} signal essentially never occurred in the fibroblasts of patients with AD, with or without a family history of AD, and occurred in some fibroblasts of every AC individual, it clearly distinguishes between individuals suffering from AD and those showing the signs of normal aging. At a minimum, therefore, the TEA-induced Ca^{2+} signal might provide the basis for an AD screening test for older populations, in much the same way that the tuberculin skin test (using purified protein derivative, PPD) usefully screens for tuberculosis (39, 40) or an ELISA test screens for AIDS (41, 42). At a maximum, the TEA-elicited Ca^{2+} signal might also provide a new means of excluding other neurologic, psychiatric, and medical disorders which now can be assessed with a variety of clinical and laboratory methods. We have shown here that, at least for some neurological and psychiatric disorders, the TEA response is normal and, therefore, the method distinguishes AD not only from normal aging but also potentially from Parkinson disease, Huntington disease, and Wernicke–Korsakoff syndrome. It should be emphasized, however, that analysis of freshly obtained cells from a variety of neurological patients as well as an expanded sample of AD patients will be necessary to more definitively establish the clinical testing value of this method. It will also be important to establish how early in the course of AD K^+ channel dysfunction appears and to determine whether it appears even prior to the onset of the disease itself.

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